GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND
VARIATION

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CORRIGENDA

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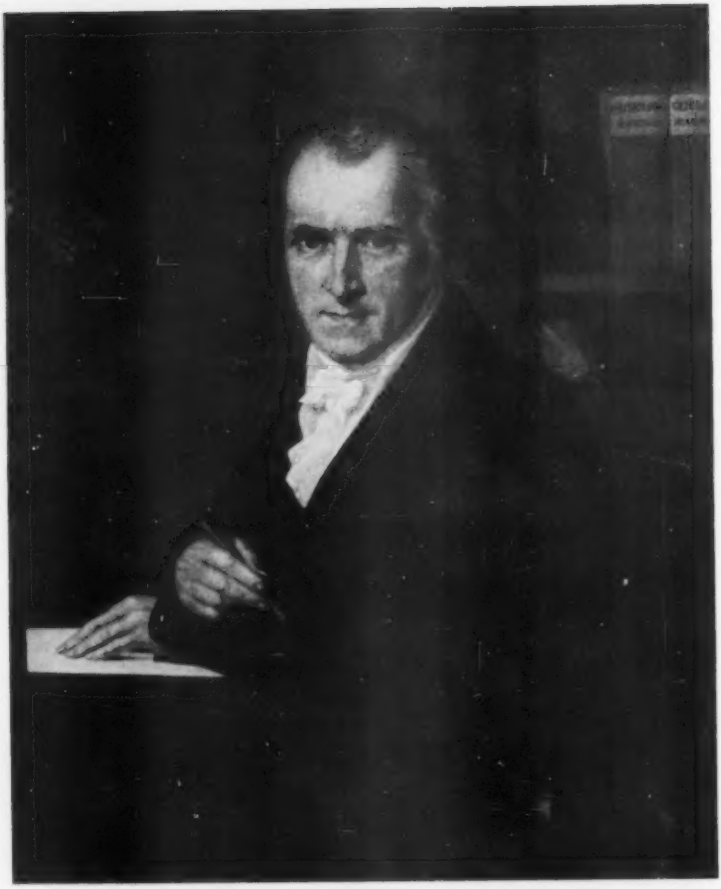
Page 160, table 6, column 4, third entry, for "38.3125" read "18.3125."
Page 160, table 6, column 5, first entry, for "31.3125" read "26.3125."
Page 160, table 6 column 5, second entry, for "31.3125" read "26.3125."
Page 160, table 6, column 5, third entry, for "31.3125" read "26.3125."
Page 160, table 6, column 5, fourth entry, for "31.3125" read "26.3125."
Page 160, table 6, column 6, first entry, for "260.25" read "255.25."
Page 160, table 6, column 6, second entry, for "45.00" read "50.00."
Page 160, table 6, column 6, third entry, for "45.00" read "50.00."
Page 160, table 6, column 6, fourth entry, for "56.75" read "51.75."
Page 160, table 6, column 4, total, for "$125.2500 \div 4 = 31.3125$" read "$105.2500 \div 4 = 26.3125$."
Page 160, line 1 under table 6, for "56.75" read "51.75."
Page 160, line 1 under table 6, for "25.32 ± 1.73" read "28.70 ± 1.85."
Page 160, line 2 under table 6, for "$\chi^2 = 9.05$; $P = 0.029$" read "$\chi^2 = 5.77$; $P = 0.137$."
Page 170, line 32, for "28.70 ± 3.43" read "22.44 ± 2.58."
Page 223, table 5, column 5, third entry, for "1.0:9" read "1.9:1."
Page 251, line 34, for "$\mp$" read "$\pm$."
Page 295, line 11, for "$+$" read "$\times$."
Page 530, table of contents, line 20, for "580" read "579."
Page 577, line 27, for "sporophyte-producing" read "sporophyte producing."



Carl Friedrich Gärtner.

KARL FRIEDRICH VON GÄRTNER

(FRONTISPIECE)

KARL FRIEDRICH VON GÄRTNER was born at Calw on May 1, 1772, and died there on September 1, 1850. His biological education was begun at the CARLSACADEMIE of Stuttgart, and was continued under the medical faculties of JENA and of GÖTTINGEN. He received his degree in 1796 and became a physician in his native town. Here he became interested in both human physiology and plant physiology and collected notes for a book on the latter subject. This work was never completed, but led to the study of sexuality in plants, which was GÄRTNER'S major interest for twenty-five years.

From these experimental studies GÄRTNER gained a lasting reputation. In 1837 he received the prize offered by the DUTCH ACADEMY at Haarlem for the best essay on sexual manifestations in plants. The published accounts of his investigations included a large volume entitled "Versuche und Beobachtungen über die Befruchtungsorgane der vollkommeneren Gewächse und über die natürliche und künstliche Befruchtung durch den eigenen Pollen." This dealt with normal, intraspecific fertilization. A later work, "Versuche und Beobachtungen über die Bastarderzeugung im Pflanzenreich" was published in 1849 and described hybridization or interspecific fertilization. At that time, GÄRTNER had already received many honors, as one notes from the title page of the volume,—Med. Dr., des K. Württ. Kr. Ordens Ritter, der Kaiserl. Leopold. Carol. Akademie der Naturforscher und Aerzte, der K. bayer. botan. Gesellschaft zu Regenspurg, der naturf. der Wetterau der des Osterlandes zu Altenburg u. m. a gelehrten Gesellschaften und Vereine Mitglied.

GÄRTNER'S work was notable for its critical spirit rather than for the wholly new principles uncovered. He confirmed conclusively the existence of sexuality in plants, which was still unaccepted by many distinguished biologists in spite of the investigations of CAMERARIUS, KÖLREUTER, KNIGHT, and others. He was a prodigious worker, having over 9000 crosses to his credit. He noted the sterility that often occurs in hybrids between species. He marked the frequent exhibition of hybrid vigor. He was also familiar with the phenomenon of self-sterility. He further found that reciprocal crosses may differ in fertility.

Throughout his career GÄRTNER'S investigations were characterized by complete detailed descriptions of morphology and of plant behavior. Such painstaking accuracy has made his papers of great value to later workers, in spite of their lack of originality.

The portrait issued as the frontispiece of this volume is from a life-size oil painting done in 1831 by the celebrated artist MORFF, who was Court Painter to FRIEDRICH, King of Württemburg. The portrait was bequeathed to the Botanical Institute of the UNIVERSITY OF TÜBINGEN by GÄRTNER'S daughter, Fräulein EMMA GÄRTNER. GENETICS is indebted to the present director of the Institute, Professor Doctor E. LEHMANN, for the photograph from which the cut was made.

VARIATIONS OF LINKAGE IN RATS AND MICE

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Bussey Institution, Harvard University, Forest Hills, Boston, Massachusetts

Received June 18, 1923

In the common brown rat (*Mus norvegicus*) there occurs a linkage system of three genes; that is, there occur in this species three characters which show with relation to one another the phenomena of coupling and repulsion in heredity. Two characters are said to be coupled in heredity when they have a tendency to stay together generation after generation, and they are said to exhibit repulsion when they have a tendency to keep apart. In general, two linked characters show coupling when they are inherited from the same parent in the same gamete and repulsion when they are inherited from different parents and so in different gametes. It is supposed that linked genes lie in the same chromosome. If this explanation is correct, it follows that in the brown rat there occur, in a common chromosome, genes for the three recessive characters, albinism, red-eyed yellow and pink-eyed yellow. With a view to learning more about the behavior of linked genes in heredity, an intensive study has been made of this case.

Everyone is familiar with the common white rat, an albino variety of *Mus norvegicus*, which has long been in captivity as a pet and is now extensively used as a laboratory animal. Its coat is practically without pigment (a creamy white) and its eyes are pink. Albinism is a simple Mendelian recessive character. Consequently albino rats produce only albino offspring.

The two yellow varieties are of more recent origin. They were discovered in England only a few years ago. They are identical with each other in appearance except for the eye color, which in one variety is similar to the albino, being practically without pigment and so showing a pink reflection from the blood in the retina. This variety is known as pink-eyed yellow. In the other variety the eye contains considerable black pigment (though less than is found in black or gray rats), consequently the eye has a dull red color and the variety is called red-eyed yellow. Fanciers call it black-eyed yellow.

[1] The observations on which this paper is based were made chiefly by the junior author; both authors have shared in the statistical analysis of the data, and the senior author has formulated this statement of results.

The yellow varieties behave in exactly the same way as the albino in crosses with the fully pigmented black or gray varieties. Each is a simple recessive character and so breeds true as soon as isolated.

When the albino variety is crossed with one of the yellow varieties, or when the yellow varieties are crossed with each other, gray or black offspring are obtained fully or nearly[2] as heavily pigmented as wild rats. This result shows that the three recessive variations are all different in nature and are really complementary.

When an F_2 generation is produced from one of these crosses it contains more of the recessive (grandparental) types and fewer of the dark type of F_1 than the percentage expected when two recessive characters are independent in their inheritance. This suggests linkage, and when more exact tests are made it is indeed found that linkage occurs in every case, though its strength is different in each case.

The linkage between albinism and red-eyed yellow is strongest of all. If an albino is crossed with a red-eyed yellow rat, gray F_1 young are obtained. These mated with each other produce an F_2 generation of albino, gray, and red-eyed yellow rats. Now if albinism and red-eyed yellow were independent (not linked), half the F_2 albinos should transmit yellow and so would produce yellow young when crossed with yellows. Also half of the F_2 yellow rats should transmit albinism and so would produce albino young when mated with albinos. In reality, we had to test several hundred F_2 albinos and red-eyed yellows before we found one that transmitted *both* albinism and red-eyed yellow. From such "cross-over" individuals a stock of double-recessive individuals was in time obtained which enabled us to determine more precisely the crossover percentage. The procedure was as follows. An F_1 individual was mated with a double-recessive individual or individuals and the young were examined at birth as to their eye color, note being made of how many had light eyes (destined to be either albinos or red-eyed yellows) and how many had dark eyes (the F_1 type). *Those having dark eyes would theoretically equal half the total number of crossover gametes produced by the F_1 parent.* For, if we designate the albino character by *c* and the red-eyed yellow character by *r*, then the gametes which unite to form the F_1 individual are *cR* and *Cr*. The F_1 individual will form gametes ordinarily of these same two types, but exceptionally (as crossovers) of the types *CR* and *cr*. The double-recessive individuals mated with the F_1 individual will produce only gametes *cr* in formula, which, united with the four kinds of gametes

[2] Crosses of the two yellow varieties with each other produce lighter grays than either cross of albino with yellow.

produced by the F_1 mate, will produce a double-dominant combination necessary for the production of dark eyes, only when united with the *CR* crossover type of gamete. In matings of F_1 with double recessive individuals, 12,587 young have been produced, of which 18 were dark-eyed, indicating the occurrence of twice 18, or 36 crossover gametes, a percentage of 0.28 (see table 1). In testing F_2 individuals for evidence of

TABLE 1

Summary of data on linkage in rats and mice.

GENES CONCERNED	GAMETES TESTED	CROSSOVER GAMETES	PERCENT CROSSOVERS	NATURE OF CROSS	SOURCE OF DATA
Albinism and red-eyed yellow in rats	434	1	0.46±1.62	Repulsion	CASTLE 1919
	270	5	1.85±2.05	Repulsion	DUNN 1920
	12,587	36	0.28±0.42	Repulsion	New data
Total	13,291	42	0.31±0.41		
Red-eyed yellow and pink-eyed yellow in rats	4,746	870	18.33±0.69	Repulsion and coupling	CASTLE 1919
Albinism and pink-eyed yellow in rats	90	19	21.10±3.55	Repulsion	CASTLE 1919
	32,735	6438	19.66±0.26	Repulsion	New data
Total	32,825	6457	19.67±0.26		
Albinism and pink-eye in mice	3,418	482	14.10±0.81	Repulsion	CASTLE 1919
	3,331	503	15.10±0.82	Coupling	DUNN 1920
	3,880	568	14.64±0.76	Coupling	New data
Total	10,629	1553	14.61±0.46		

crossing over (CASTLE 1919, DUNN 1920), in the preliminary work, the occurrence was noted of 6 crossovers among 704 gametes which entered into the production of 352 tested individuals. This is less than one percent of crossovers. Combining the data from the F_2 tests, with those obtained from back-crosses, we have 42 indicated crossovers in 13,291 gametes, or 0.31 ± 0.41 percent, which in round numbers is one-third of one percent of crossovers. The numbers are large enough to show that crossing over between these two genes is a rare event.

A word might be said as to the statistical significance in this case of the calculated crossover percentage, which is slightly less than its probable

error. Does this mean that doubt exists as to the occurrence of crossing over between the two genes? By no means. The demonstration of the existence of a single F_2 individual, which transmitted in the same gamete both yellow and albinism, would constitute incontrovertible proof that crossing over had occurred. Six such individuals were obtained. The probable error in this case indicates merely the likelihood that, on the repetition of the experiment, crossovers would again be obtained in comparable numbers.

The probable errors throughout this paper are based on the convenient tables prepared and distributed privately by Dr. R. A. EMERSON in 1922. In the use of these tables in our work, the probable error has been based, not as ordinarily on the total population studied, but on *half* the total population, for the reason that the observed number of crossovers was really that of the half population, since one of the two theoretically equal classes of crossovers was not identified. This procedure increases considerably the probable error, over what it would otherwise be in populations of the size recorded.

Between the genes for pink-eyed yellow and for red-eyed yellow, the crossover percentage is 18.33 ± 0.69 (data of CASTLE 1919), based on tests of 4746 gametes produced by F_1 males and females.

The linkage is least between the genes for albinism and pink-eyed yellow. Back-crosses of F_1 animals of both sexes with double recessive individuals have been studied very extensively in this case. They indicate that of 32,735 gametes produced by F_1 animals, 6438 were crossovers. This is a crossover percentage of 19.66 ± 0.26. Combining with these figures data reported by CASTLE (1919) on tests of 90 F_2 animals, which gave an indicated crossover percentage of 21.10 ± 3.55, we have for this linkage relation, data on 32,825 gametes produced by F_1 animals, giving a crossover percentage of 19.67 ± 0.26.

These figures clearly indicate that the order of the genes, if linear, is (1) albinism, (2) red-eyed yellow and (3) pink-eyed yellow, since the greatest crossover percentage occurs between albinism and pink-eyed yellow, which makes them the end genes of the series. Before we discuss the evidence for the linearity or non-linearity of the arrangement of the three genes, it will be necessary to consider the relation of sex to the crossover percentage, since in the data already given in summary fashion, male and female parents were unequally represented.

In all the rat linkages, as also in the linkage studied in mice, F_1 females give a higher crossover percentage than males give. See table 2. In the case of albinism and red-eyed yellow, F_1 females have given a crossover

TABLE 2

Data on the frequency of crossing over in the two sexes of rats and mice.

GENES CONCERNED	SEX OF PARENT	GAMETES TESTED	CROSSOVER GAMETES	PERCENT CROSSOVERS	NATURE OF CROSS	SOURCE OF DATA
Albinism and red-eye in rats	Female	3,759	20	0.53±0.78	Repulsion	New data
	Male	8,828	16	0.18±0.50	Repulsion	New data
Total		12,587	36	0.28±0.42		
Red-eye and pink-eye in rats	Female	2,683	549	20.46±0.92	Repulsion and coupling	CASTLE 1919
	Male	2,063	321	15.55±1.04	Repulsion and coupling	CASTLE 1919
Total		4,746	870	18.33±0.69		
Albinism and pink-eye in rats	Female	11,480	2518	21.93±0.44	Repulsion	New data
	Male	21,255	3920	18.39±0.32	Repulsion	New data
Total		32,735	6438	19.66±0.26		
Albinism and pink-eye in mice	Female	2,789	444	15.92±0.90	Repulsion and coupling	DUNN 1920
	Male	3,683	503	13.65±0.78	Repulsion and coupling	DUNN 1920
	Female	556	106	19.06±2.02	Coupling	New data
	Male	3,324	462	13.89±0.82	Coupling	New data
	Female	3,345	550	16.44±0.82		Combined data
	Male	7,057	965	13.77±0.57		Combined data
Total		10,352	1515	14.63±0.47		

percentage of 0.53±0.78, while males have given 0.18±0.50 percent. In the cross of albino with pink-eyed yellow, F_1 females have given 21.93±0.44 percent of crossovers, whereas males have given 18.39±0.32, a difference of more than three percent between the sexes. As regards the relation of pink-eyed yellow to red-eyed yellow, F_1 females have given 20.46±0.92 percent crossovers, and males have given 15.55±1.04 percent. The probable errors are greater in the last case than in either of the others because fewer young were produced.

The data obtained from mice are entirely consistent with those given by rats. In the cross of albino with pink-eyed colored mice, F_1 females have given a crossover percentage of 16.44 ± 0.82 and F_1 males 13.77 ± 0.57 percent, a difference of nearly three percent. All the data are consistent with the view that in rats and mice a pair of chromosomes containing genes for albinism and pink-eyed colored coat cross over somewhat more freely in females than in males. This being the case, it is legitimate to compare only crossover percentages obtained from the same sex.

On the theory of linear arrangement of the genes, the crossover percentage between the most distant genes in a linkage system should approximate the sum of the intermediate linkages from gene to gene. That is, AC should equal $AB+BC$, when the order of the genes is ABC. The facts in the present case are in harmony with this theory. Calling the three genes c(albinism), r (red-eyed yellow), and p (pink-eyed yellow), we should have $cr+rp=cp$. Substituting the observed, we have for males, $0.18+15.55=18.44$, which is greater than the actual sum of the first two terms (15.73) by 2.71 percent, but the sum of the three probable errors is 1.86 percent, more than half of the observed deviation. In the case of females the equation would read $0.53+20.46=21.93$, which again is greater than the true sum of the first two terms (20.99), although the discrepancy is not so great, being 0.94, the sum of the three probable errors being 2.14. In each case $AC>AB+BC$, yet the difference is no greater than random sampling might produce. The linear arrangement theory is tenable in the case.

A literal interpretation of the chromosome theory might lead one to the conclusion that in this case homologous chromosomes are *longer* in females than in males, but such a suggestion can scarcely be considered seriously. There is a difference in the physiology of maturation in ova and in spermatogonia. In Drosophila *no* crossing over occurs in males, though the chromosomes certainly have appreciable length in the male. On the contrary, in silk-moths crossing over occurs *only* in males, though the chromosomes are probably quite as long in the egg as in the sperm-cell. In rats and mice it is evident, at least in the case of one pair of chromosomes, that crossing over occurs a little more freely in females than in males, but there is no reason to think that the arrangement or spacing of the genes is different in the two sexes.

Aside from sex, we have investigated two other factors which might conceivably influence the amount of crossing over, namely, the age of the parent, and seasonal conditions, in particular, temperature. Both these investigations have yielded negative results. There was some indication

of a rise in the crossover percentage in litters born in the warmer months, July, August and September, but fuller investigation failed to substantiate the suggestion. Age of the parent and size of the litter also exert no discoverable effect on the crossover percentage. Differences in the crossover percentages given by different male F_1 individuals have also been sought but largely without success. The crossover percentages of individual male parents fluctuate in a typical curve of error, and selection of extreme individuals has had no racial effect. Hence, we conclude that such fluctuation has no genetic significance, but is due solely to random sampling.

TABLE 3

Crossover percentages between albinism and pink-eyed yellow among the young sired by males of known age in months.

AGE OF SIRE IN MONTHS	YOUNG WITH LIGHT EYES	YOUNG WITH DARK EYES	TOTAL YOUNG	PERCENT CROSSOVERS	P. E.	DEVIATION FROM AVERAGE	DEV. / P. E.
3	880	78	958	16.28±	1.54	−1.81	1.17
4	1963	194	2157	17.98±	1.02	− .11	.10
5	2081	209	2290	18.24±	.99	+ .15	.15
6	2029	215	2244	19.16±	1.00	+1.07	1.07
7	2220	249	2469	20.17±	0.96	+2.08	2.16
8	1838	187	2025	18.47±	1.05	+ .36	.34
9	1658	181	1839	19.68±	1.11	+1.59	1.43
10	1302	126	1428	17.64±	1.24	− .45	.36
11	1231	104	1335	15.58±	1.31	−2.51	1.91
12	927	81	1008	16.07±	1.50	−2.02	1.34
13	759	60	819	14.65±	1.67	−3.44	2.06
14	567	64	631	20.28±	1.90	+2.19	1.15
15	400	30	430	13.95±	2.30	−4.04	1.74
16	263	32	295	21.69±	2.78	+3.60	1.29
17	176	10	186	10.75±	3.50	−7.34	2.97
18	69	4	73	10.95±	5.55	−7.14	1.28
19	29	3	32	18.75±	8.43	+ .66	.08
20	22	3	25	24.00±	9.50	+5.91	.62
21	5	2	7				
Total	18,419	1832	20,251	18.09	0.33		

Table 3 shows the crossover percentage among the young sired by F_1 males of known age from three to twenty-one months old. The average crossover percentage among the 20,251 young, was 18.09 ± 0.33. It was slightly lower than the average when the sires were three and four months old, then above the average for the next five months, again below the average for the next four months, and then oscillated between high and low average values. No consistency is shown in the deviations for young

or old sires. In no month does the deviation from the general average reach three times the probable error. In only three months does it exceed twice the probable error, and these are widely scattered, being in the seventh, thirteenth and seventeenth months, respectively. Accordingly, the observed deviations are without statistical significance, being satisfactorily accounted for as the results of random sampling alone.

TABLE 4

Crossover percentages between albinism and pink-eyed yellow among the young of F_1 females of known age in months.

AGE IN MONTHS	DARK-EYED YOUNG	LIGHT-EYED YOUNG	TOTAL YOUNG	PERCENT CROSSOVERS	P. E.	DEVIATION FROM AVERAGE	DEV. / P. E.
3	435	71	506	28.06	2.12	+6.50	3.06
4	920	110	1030	21.36	1.49	−0.20	0.14
5	1260	121	1381	17.52	1.28	−4.04	3.15
6	986	117	1103	21.21	1.44	−0.35	0.24
7	1148	157	1305	24.06	1.32	+2.50	1.89
8	961	117	1078	21.70	1.45	+ .14	0.09
9	834	108	942	22.92	1.55	+1.36	0.90
10	665	84	749	22.43	1.74	+0.87	0.50
11	600	67	667	20.09	1.85	−1.47	0.79
12	420	50	470	21.27	2.20	−0.29	0.13
13	407	34	441	15.42	2.27	−6.14	2.70
14	327	44	371	23.72	2.47	+2.46	0.87
15	188	24	212	22.64	3.28	+1.08	0.32
16	161	24	185	25.94	3.51	+4.38	1.24
17	91	9	100	18.00	4.77	−3.56	0.74
18	47	5	52	19.23	6.61	−2.33	0.35
19	5	1	6				
20	5	0	5				
Total	9460	1143	10,603	21.56	0.46		

Table 4 contains similar data on the crossover percentage given by F_1 females. Neither old nor young females give percentages consistently high or low. The deviations from the average crossover percentage reach three times the probable error in two months and exceed twice the probable error in a third month, but are of doubtful statistical significance in any case.

Table 5 shows the variation in crossover percentage among individual F_1 parents. Sixty-one different males included in this table produced from 30 to 591 young each. The crossover percentages among these young range from 9.03 to 32.42, average 18.39 ± 0.32. The deviations from the average are less than the probable error in 31 cases, lie between once

TABLE 5

Summary of the young sired by individual F_1 males heterozygous for albinism and pink-eyed yellow.

MALE BRED IN CAGE NUMBER	TOTAL YOUNG	PERCENT CROSSOVERS	P. E.	DEVIATION FROM AVERAGE	DEV. / P. E.
151	83	28.91	5.20	+10.52	2.02
153	387	24.28	2.42	+ 5.89	2.43
155	548	17.51	2.04	− 0.88	0.43
157	451	18.18	2.24	− 0.21	0.09
159	245	19.59	3.04	+ 1.20	0.39
183	62	22.58	6.06	+ 4.19	0.69
185	498	20.40	2.14	+ 2.01	0.93
187	251	17.52	3.00	− 0.87	0.29
189	535	16.82	2.06	− 1.57	0.76
191	389	14.91	2.41	− 3.48	1.44
193	387	21.18	2.42	+ 2.79	1.15
195	509	16.89	2.11	− 1.50	0.71
200	166	24.09	3.70	+ 5.70	1.64
241	522	13.02	2.09	− 5.37	2.56
243	410	24.87	2.36	+ 6.48	2.73
245	177	18.07	3.57	− 0.32	0.09
247	376	15.42	2.46	− 2.97	1.20
249	386	15.02	2.43	− 3.37	1.38
251	425	14.11	2.31	− 4.28	1.85
253	291	21.30	2.79	+ 2.91	1.04
255	376	19.14	2.46	+ 0.75	0.30
257	351	21.65	2.54	+ 3.26	1.28
259	487	19.30	2.16	+ 0.91	0.42
261	504	16.66	2.12	− 2.33	1.09
265	367	13.62	2.49	− 4.77	1.91
267	227	23.78	3.16	+ 5.39	1.70
269	433	19.87	2.29	+ 1.48	0.64
271	214	23.37	3.26	+ 4.98	1.52
273	502	19.12	2.13	+ 0.73	0.34
275	231	18.18	3.13	− 0.21	0.06
277	361	17.72	2.51	− 0.67	0.26
279	362	23.20	2.51	+ 4.81	1.90
303	432	18.98	2.29	+ 0.59	0.25
305	241	22.40	3.07	+ 4.01	1.30
307	307	18.89	2.72	+ 0.50	0.18
309	331	18.12	2.62	− 0.27	0.10
311	305	14.42	2.73	− 3.97	1.45
313	177	9.03	3.57	− 9.36	2.62
315	478	23.43	2.18	+ 5.04	2.31
329	262	13.74	2.95	− 4.65	1.57
331	365	17.53	2.49	− 0.86	0.34
333	309	18.12	2.71	− 0.27	0.10
335	367	13.62	2.49	− 4.77	1.91
337	451	16.85	2.24	− 1.54	0.68
341	287	12.54	2.81	− 5.85	2.08
343	30	13.33	8.71	− 5.06	0.58

TABLE 5 (continued)

Summary of the young sired by individual F_1 males heterozygous for albinism and pink-eyed yellow.

MALE BRED IN CAGE NUMBER	TOTAL YOUNG	PERCENT CROSSOVERS	P. E.	DEVIATION FROM AVERAGE	DEV. / P. E.
343[a]	90	15.55	5.03	− 2.84	0.56
345	323	19.19	2.65	+ 0.80	0.30
347	551	21.41	2.03	+ 3.02	1.48
349	591	17.25	1.96	− 1.14	0.58
351	562	17.08	2.01	− 1.31	0.65
353	230	11.30	3.14	− 7.09	2.25
355	342	16.95	2.58	− 1.44	0.55
357	184	16.30	3.52	− 2.09	0.59
359	298	26.17	2.76	+ 7.78	2.81
361	581	14.11	1.98	− 4.28	2.16
363	371	18.86	2.47	+ 0.47	0.19
365	305	13.11	2.73	− 5.28	1.93
369	475	32.42	2.19	+14.03	6.40
263	341	15.83	2.58	− 2.56	0.99
493	137	17.51	4.06	− 0.88	0.21
Total	21,324	18.39	0.32		

TABLE 6

Summary of the young produced by groups of 3 or 4 F_1 females heterozygous for albinism and pink-eyed yellow.

REARED IN CAGE NUMBER	TOTAL YOUNG	PERCENT CROSSOVERS	P. E.	DEVIATION FROM AVERAGE	DEV. / P. E.
161	219	9.13	3.22	−12.56	3.90
162	252	27.72	3.00	+ 6.03	2.01
163	220	21.81	3.22	+ 0.12	0.03
164	312	28.20	2.70	+ 6.51	2.41
165	174	12.74	3.62	− 8.95	2.47
166	208	21.15	3.31	− 0.44	0.13
167	252	19.84	3.00	+ 0.15	0.05
168	119	20.16	4.35	− 1.53	0.35
169	127	23.62	4.22	+ 1.93	0.45
170	222	19.81	3.20	− 1.88	0.58
171	505	24.55	2.12	+ 2.86	1.34
172	208	19.23	3.31	− 2.46	0.74
174	193	24.87	3.42	+ 3.18	0.92
175	228	21.05	3.16	− 0.64	0.20
176	190	24.21	3.46	+ 2.52	0.72
177	272	22.05	2.89	+ 0.36	0.12
178	225	28.44	3.17	+ 6.75	2.13
179	183	19.67	3.52	− 2.02	0.57
180	249	17.67	3.02	− 4.02	1.33
281	174	19.54	3.62	− 2.15	0.59
282	253	15.01	2.99	− 6.68	2.23
283	112	14.28	4.51	− 7.41	1.64

TABLE 6 (continued)

Summary of the young produced by groups of 3 or 4 F_1 females heterozygous for albinism and pink-eyed yellow.

REARED IN CAGE NUMBER	TOTAL YOUNG	PERCENT CROSSOVERS	P. E.	DEVIATION FROM AVERAGE	DEV. / P. E.
284	84	14.28	5.20	− 7.41	1.42
285	225	24.00	3.17	+ 2.31	0.73
286	88	11.36	5.08	−10.33	2.03
287	48	16.66	6.88	− 5.03	0.73
288	301	21.26	2.74	− 0.43	0.15
289	59	20.33	6.16	− 1.36	0.22
290	323	24.42	2.65	+ 2.73	1.03
291	82	24.39	5.27	+ 2.70	0.51
292	106	7.54	4.63	−14.15	3.05
295	185	16.21	3.50	− 5.48	1.56
296	303	25.08	2.74	+ 3.39	1.23
297	265	22.66	2.92	+ 0.97	0.33
298	183	22.95	3.52	+ 1.26	0.35
299	109	22.01	4.55	+ 0.32	0.07
300	302	21.19	2.74	− 0.50	0.18
301	252	23.80	3.00	+ 2.11	0.70
302	112	17.85	4.51	− 3.84	0.85
318	219	23.74	3.22	+ 2.05	0.63
319	185	24.86	3.50	+ 3.17	0.90
319[a]	63	12.69	5.96	− 9.00	1.51
320	332	15.66	2.62	− 6.03	2.30
325	230	21.73	3.14	+ 0.04	0.01
333	69	26.08	5.70	+ 4.39	0.77
371	338	22.48	2.59	+ 0.79	0.30
372	294	25.16	2.78	+ 3.47	1.24
373	287	20.20	2.47	− 1.49	0.60
374	140	17.14	4.03	− 4.55	1.12
375	140	15.71	4.03	− 5.98	1.48
376	68	23.52	5.78	+ 1.83	0.31
377	68	29.41	5.78	+ 7.72	1.33
378	281	32.02	2.84	+10.33	3.63
379	253	28.45	2.99	+ 6.76	2.26
380	295	22.37	2.77	+ 0.68	0.24
Total	11,186	21.69	0.45		

and twice the probable error in 19 cases, lie between twice and three times the probable error in 10 cases, and exceed three times the probable error in only one case, that of the male bred in cage 369 which sired 475 young. He is credited with a very large number of crossovers, 6.4 times the probable error. His case alone suggests a possibly significant deviation from the average. As soon as it was recognized that this male was giving an unusually high crossover percentage, he was given additional mates and subsequent litters of young were examined with especial care. It

was found that he was heterozygous, not for ordinary albinism but for its allelomorph, ruby-eye, and one of his double-recessive mates also contained one dose of ruby-eye with one of ordinary albinism. Homozygous ruby-eyed young resulted from this mating and it is possible that in the earlier litters, which were destroyed at birth, some of these ruby-eyed young may have been classed as dark-eyed. Accordingly, all litters of this female were excluded from the total, but still the percentage of dark-eyed young credited to this male was abnormally high. To see whether the peculiarity of this male would be inherited by his descendants, several of his doubly heterozygous sons were mated with double recessive females. Such matings produced a total of 351 young with an indicated crossover percentage of 17.37 ± 3.00, a perfectly normal result. Apparently the male had not transmitted his peculiarity to any of his sons. The question whether ruby-eye gives a crossover percentage different from that of its allelomorph, ordinary albinism, is being investigated further.

An F_1 male which in his earlier litters had given only seven percent of crossovers was also made an object of special study, but two months later the percentage had risen to fifteen and it showed subsequently no tendency to return to its earlier low amount. Random sampling seems an adequate explanation of the peculiar early result.

SUMMARY

1. Three genes of the common rat, which belong to a common linkage system, are apparently linear in their arrangement. The order of the genes is *crp*.

2. The crossover percentages for these genes are regularly higher among the gametes of F_1 females than of F_1 males. The same is true for two linked genes of the house mouse, possibly homologous with genes *c* and *p* of the rat.

3. No evidence has been obtained that the crossover percentage varies with age of the parent, or temperature or other seasonal conditions, either in rats or in mice.

4. Variations in crossover percentages given by different male parents are capable of explanation as due to chance alone, except possibly in the case of one individual which, however, did not transmit the peculiarity to his sons.

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STUDIES ON SELF-STERILITY VI. THE GENETIC BASIS OF CROSS-STERILITY IN NICOTIANA[1]

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INTRODUCTION

The first papers of this series, reporting work done at the Bussey Institution of HARVARD UNIVERSITY between 1910 and 1917, left unsettled a number of questions relating to the genetic behavior of self-sterile plants. It will be remembered that studies of hybrids between *Nicotiana alata* Lk. and Otto var. *grandiflora* Comes[2] and *Nicotiana Forgetiana* (Hort.) Sand. revealed the presence of cross-sterility of surprisingly regular behavior. Each of the populations studied could be divided, by means of its cross-sterility relationships, into a number of inter-fertile, intra-sterile classes. This may be somewhat clearer if we take a concrete example. The population known as family H was found to consist of three inter-fertile, intra-sterile classes which were arbitrarily named A, B and C. All of the members of each class were sterile with all the other members of that class but were fertile with the members

[1] One of a series of papers submitted to the faculty of the Bussey Institution of HARVARD UNIVERSITY in partial fulfillment of the requirements for the degree of Doctor of Science.

[2] Hereafter *N. alata grandiflora* will for convenience be referred to as *N. alata*.

of the other two classes. No plant in class A, for example, would set seed when pollinated with pollen from another plant in that same class, though it would set an abundance of seed when pollen from plants in classes B and C was applied. In other words, each class behaved as if it were a single self-sterile individual.

Upon examining the material histologically it was found that pollen germinated normally when applied to the stigmas of the plant on which it was produced and that the pollen-tubes grew down the style at a constant rate of speed. Under normal conditions this growth was not fast enough to allow them to reach the ovule during the "life" of the flower, and therefore no seed was obtained. Precisely the same thing occurred when the pollen was applied to the stigmas of other plants in the same class. When, however, it was applied to the stigmas of plants with which it was cross-fertile, the pollen-tube grew at an increasing rate and reached the ovule in time to cause fertilization and subsequent seed production. These phenomena were interpreted as due to the interaction of various germinal factors. According to this view, plants which were cross-sterile with each other had certain essential factors in common; plants which were cross-fertile with each other differed for some of these factors. If two plants, and hence the pollen-tube and the style, differed for these factors, a stimulus was produced which caused the accelerated growth of cross-fertile matings. When, however, as in a self-pollination or a cross-sterile pollination, the pollen-tube and the style had the same factors, there was no stimulus, no accelerated growth, and fertilization did not occur. A class, according to this view, was made up of a number of plants possessing certain essential factors in common. The present investigation was undertaken to determine the number of distinct classes in the material at hand and to study the manner of their inheritance. While it was not certain that the same system of inter-fertile, intra-sterile classes operated in *N. alata* and *N. Forgetiana* as in their hybrids, there were reasons for believing this to be the case. It was planned to determine this definitely; to find out if there were any classes common to both species; and to investigate the relations between the classes of the two species and those of their hybrids.

MATERIALS AND METHODS

Owing to the exigencies of war work, much of the seed from the earlier cultures on hand was dead when the present writer took up the problem in April 1919. Because of this unfortunate but unavoidable circumstance the work could not be prosecuted as rapidly as had at first been thought possible and it was some time before the project was well under way.

Two species of Nicotiana were used, *N. Forgetiana* (Hort.) Sand. and *N. alata* Lk. and Otto var. *grandiflora* Comes. As has already been explained by EAST (1913), the *N. Forgetiana* used throughout the work with self-sterility had its origin in seeds obtained from SANDER AND SONS under the name of *N. Sanderae*. *Nicotiana Sanderae* (*N. alata* × *N. Forgetiana*) is very variable in color, the flowers ranging from white through pink and magenta to purple and crimson. In size they vary greatly, although they are uniformly larger than those of *N. Forgetiana*. The plants here discussed were constant through five generations for flower size, color and form and showed not the slightest trace of *N. alata* characteristics. They were, moreover, the exact duplicates of SANDER'S figure of *N. Forgetiana* published in the Botanical Magazine (No. 8006) and we therefore feel justified in supposing that they originated from seeds of pure *N. Forgetiana* which had been inadvertently mixed with the hybrid *N. Sanderae* seed.

The material was well suited to our needs. It grew splendidly under greenhouse conditions and plants could be brought into flower at practically any season of the year. Single plants could be cut back and made to flower again and again. Ninety-five percent of compatible matings were successful and there was an abundance of seed from each mating. The chief advantage for the present investigation was that the germinal factors affecting self-sterility and self-fertility were much stronger than the environmental ones. It was therefore excellent material in which to attempt to trace the course of germinal factors. Had it been otherwise, cramping the plants in pots and growing them under artificial conditions might have so obscured the effect of the germinal factors that they could not have been studied. Or if, as in many other species, the normal percentage of successes in compatible crosses had been only 50 to 75 instead of 95 it would have been much more difficult to determine whether any two plants were cross-fertile or cross-sterile.

Every precaution was taken to reduce the possibility of experimental error. All seed was planted in sterile soil. All pollinations were made upon emasculated flowers which were inclosed in paper bags. During the last few months' work when large numbers of plants were being classified, these precautions were not observed, but this was during the winter when there were no insects in the greenhouse. At all other times pollen was used only from newly-opened, bagged flowers. Whenever there was any reason to believe that pollen from nearby plants might be a source of contamination all these flowers were destroyed before the paper bag was removed. In emasculating and pollinating, the hands and instruments were washed

in 95 percent alcohol after each operation to destroy any foreign pollen. The efficacy of this method was tested in the following manner; The fingers were dusted with pollen and four pollinations were made. The hands were then rinsed very lightly with alcohol. When the remaining pollen (of which there was a liberal amount) was thoroughly dry, four additional pollinations were made on the same plant. No seeds were produced from these later pollinations, but all those made before the application of alcohol resulted in plump, well-filled capsules.

The work was done under the direction of Professor E. M. EAST to whom the writer is indebted for advice and encouragement. He is also under obligations to Miss DOROTHY MOORE for assistance with the manuscript.

REVIEW OF RECENT LITERATURE

Since the earlier papers of this series were written several interesting articles on the subject have appeared. BAUR (1919) investigated the self-fertility of several species of the Antirrhinastrum section of the genus Antirrhinum and corroborated many of EAST and PARK's (1917) observations. *Antirrhinum siculum* and a wild Spanish form of *A. majus* were fully self-fertile and set seed as well with their own pollen as when cross-pollinated. *A. tortuosum*, *A. latifolium*, and two wild forms of a Portuguese species were found to be self-sterile during the first year, although they were slightly self-fertile toward the end of the blooming season in the fall, a condition paralleled in Nicotiana by the end-season fertility reported by EAST and PARK. Unlike Nicotiana, however, these species did not regain their self-sterility with the advent of another blooming period but were very self-fertile the second year. *A. Ibanyezii*, *A. molle*, *A. glutinosum*, *A hispanicum*, and an undetermined Spanish species were found to be fully self-sterile.

Like COMPTON (1912) and EAST and PARK (1917) BAUR found that crosses between self-sterile species gave self-sterile hybrids and that in crosses between self-fertile and self-sterile species, self-fertility behaved as a dominant. BAUR obtained an F_2 which suggested a 15 : 1 ratio and this conclusion was further borne out by the fact that the self-fertile plants differed among themselves in the expression of their self-fertility, suggesting the presence of a two-factor difference. While EAST and PARK obtained a 3 : 1 ratio, there were certain differences among the self-sterile plants which suggested an additional factor; in other words a 12 : 3 : 1 ratio, a result quite in accord with BAUR's. While BAUR was not able to investigate cross-sterility as extensively as EAST and PARK

(due in part to the nature of his material), his findings are similar. In a cross between two plants of *A. hispanicum* 31 seedlings were obtained. These could be divided by their cross-sterility and cross-fertility relationships into two inter-fertile, intra-sterile groups, approximately equal in size. One group was fertile with both parents, one sterile with one parent but fertile with the other. This is remarkably like the result reported in the present paper, obtained by crossing two plants of *N. Forgetiana*.

LEHMANN (1919) studied the self-sterile species, *Veronica syriaca*. He found inter-fertile, intra-sterile classes, pseudo-fertility, and identical behavior of reciprocal crosses as regards cross-sterility. He had been conducting experiments on this species for several years. During that time he had not found a single self-fertile individual, and had noticed that certain combinations were repeatedly cross-sterile. Accordingly a cross was made between two plants, and a first generation of 198 plants was grown. All were perfectly self-sterile. Of these, 32 were chosen for intensive experiment and 983 of the possible 992 combinations were effected, 233 pollinations being made. The 32 plants could be divided into four intra-sterile, inter-fertile classes, A, B, C and D. Of the many crosses between plants of different classes, 97.5 percent were fertile; of the many between plants of the same class only 1.1 percent set seed and these were small abortive capsules. After these four groups had been determined, crosses were made with the remainder of the first-generation plants in order to classify as many of them as possible. The relationships of 131 plants were determined. They fell into four classes in the following numbers: A, 35; B, 27; C, 37; D, 31.

As *V. syriaca* is neither wild nor cultivated in the region where LEHMANN was working, it was possible to test the cross-sterility of the classes in a very severe manner by growing representatives of each class in isolated plots and allowing insects to make the pollinations. Several plants of each class were isolated, 13 A's, 10 B's, 21 C's, and 12 D's. Of the 8964 flowers recorded on these 56 plants, 8785, or practically 98 percent, set no seed. The other 2 percent for the most part formed small abortive capsules. In fact only 5 seeds were obtained from all the plants. A check plot of plants from all the classes set an abundance of seed.

DEVRIES (1906) seems to have been the first to notice inter-fertile, intra-sterile classes among self-sterile plants. In a short note on *Linaria vulgaris* quoted by CORRENS (1916), he says:

". . . dass es zweierlei Arten von Löwenmaul gibt, die man allerdings von aussen nicht unterscheiden kann, die aber doch verschieden sind. Bei jedem Typus sind die einzelnen Pflanzen unter sich unfruchtbar, vereinigt man jedoch zwei Pflanzen, die

verschiedenen Typen angehören, dann gibt es eine normale und reichliche Ernte."

DEVRIES apparently went no further with the matter and there it rested until CORRENS started working with the genus in 1913. He obtained 2 plants of *Linaria vulgaris* from different localities and crossed them. Unfortunately these parent plants died before the next generation came into flower, and it was possible to test the 13 plants of the next generation only among themselves. There were 4 classes, all inter-fertile and intra-sterile, except that class D was fertile with class B when used as a male parent and sterile when used as a female. The number of plants and the number of pollinations are so small that it is difficult to say whether the same sort of thing is in operation here as in Antirrhinum, Veronica and Nicotiana, or whether there are really some minor differences. CORRENS described the phenomenon which EAST (1917) has called pseudo-fertility. He also noted that several cross-sterile matings yielded small abortive capsules.

SIRKS (1917) found quite a different condition in *Verbascum phoenicium*, where conditions are further complicated by the presence of true functional sterility. There was much cross-sterility but no regularity which would permit grouping into classes. Reciprocal crosses very often gave different results. There seemed to be a tendency towards dioecism and the plants could be arranged in a graded series. The extremes at one end of the series usually (about 3 times out of 4) are fertile when used as a female parent, but sterile when used as a male. At the other end are plants which seldom are fertile as females but are nearly always fertile as males.

STOUT (1920) has continued his earlier work (1916) on self-sterility and self-fertility in *Cichorium Intybus* and has made preliminary investigations in several other genera. Before considering his work it will be necessary to define certain terms. If a plant sets abundant seed with its own pollen it is called self-fertile. If it sets no seed at all it is called self-sterile. In describing these two extremes all writers are in agreement. Among self-sterile plants, however, there will be found individuals which are very slightly self-fertile. This fertility may exhibit itself as a swelling of the capsule with few or no seeds produced; in only a few cases will it approach to anything like the fertility of self-fertile plants. EAST (1917), working on Nicotiana, showed that it was a variant of self-sterility and named the phenomenon "pseudo-fertility." A similar condition has since been reported from *Veronica syriaca* by LEHMANN (1919), from *Linaria vulgaris* by CORRENS (1916), from Antirrhinum by BAUR (1919), and STOUT himself has described its occurrence in several species.

STOUT has used no term to distinguish between perfect self-fertility and this pseudo-fertility and one has to judge from his data which is meant. When he says, for instance, (STOUT 1917, p. 387) that 10 of 101 offspring of two self-sterile plants were self-fertile, one finds by turning to table 8 on pages 381-383 that the percentages of self-fertility of these plants are as follows: 6 percent, 13 percent, 63 percent, 3 percent, 11 percent, 50 percent, 4 percent, 71 percent and 73 percent. Not one of them is perfectly self-fertile, not more than two or three of them even approach to a self-fertility comparable with that of self-fertile plants, and yet they are all described as self-fertile! In the same way he has failed to distinguish between true cross-fertility and pseudo-cross-fertility. His failure to do this undoubtedly lies in the nature of his material. The pseudo-fertility of *Cichorium Intybus* is very variable, much more so than that of *Eschscholtzia californica*, for instance. In some cases it very nearly approaches to true fertility,—just how nearly cannot be calculated, since STOUT gives no complete data for crosses that are unmistakably compatible. The high percentage of failures to set seed (as compared with the percentage in Nicotiana for instance) is another factor which has undoubtedly tended to obscure the difference between true fertility and pseudo-fertility.

STOUT finds no end-season fertility (or progressive increase in pseudo-fertility) in *Cichorium Intybus*. While he comes to no definite conclusions as to the inheritance of self-sterility, he seems to recognize the presence of a germinal basis. In speaking of self-fertility and self-sterility he says (STOUT 1920, p. 122),

> "Nevertheless, there is a tendency to heritability in these characters. Self-fertile plants[3] appear to constitute from 0 to 10 percent of the progeny of self-sterile plants. . . . . The offspring of self-fertile plants are more likely to be self-fertile by a proportion of about 5 to 1."

He also goes on to say that this proportion can be changed slightly by selection.

In *Verbascum phoeniceum*, STOUT, like SIRKS (1917), found a few individuals which were almost completely sterile as males but fertile as females. Upon examination 95 percent of the pollen was found to be shrivelled and empty, showing that there is functional sterility in addition to self-sterility.

Eschscholtzia californica was found to have little pseudo-fertility. Only 1 plant out of nearly 200 grown had a self-fertility comparable with the fertility in compatible crosses. Of these plants, 128 were completely

[3] Bear in mind that STOUT includes pseudo-fertile plants under the term self-fertile plants.

self-sterile, 43 bore small seedless pods, 24 had small pods with few seeds. There was no end-seasonal fertility.

Raphanus sativus was even more strongly self-sterile. Only 1 plant out of the nearly 200 grown showed any signs of pseudo-fertility.

In *Brassica pekinensis* a few of the plants showed a mid-seasonal fertility. *Linum grandiflorum* (a heterostyled species) was self-sterile and the same forms were inter-sterile.

SHULL (1923) has recently reported on the self-sterile species *Bursa grandiflora* Bois. He finds that this species is slightly pseudo-fertile and that his cultures are made up of two inter-fertile, intra-sterile classes.

PRESENTATION OF DATA

Nicotiana Forgetiana (Hort.) Sand.

Family AB (open-pollinated *N. Forgetiana*)

Nearly all the seed of *Nicotiana Forgetiana* on hand was dead; the one lot which germinated was an open-pollinated capsule which had been gathered from a field-grown plant at the end of the season. Nineteen plants were raised from this capsule and designated as family AB. One died before coming into flower. Two were strikingly different from the rest and were identical with the hybrids which had previously been made between *N. Forgetiana* and *N. alata*. It may be well to add that hybrids had been raised between *N. Forgetiana* and all the species with which it would cross and that they were all easily recognized. Inasmuch as there was every opportunity for natural crossing between these two species in the field in which the mother plants were grown, there is no reason for believing that these two plants were anything but natural hybrids between *N. Forgetiana* and *N. alata*. They were sterile with each other and fertile with all other plants with which they were crossed. The other 16 plants were all uniform. They possessed the short, deep-red flowers characteristic of *N. Forgetiana* and their corolla length accorded with that previously reported for the species by EAST (1913).

Inasmuch as the procedure followed in studying this family is typical of that followed throughout the whole investigation, it may be well to give it in some detail. In beginning the work, cross-pollinations were made between these different plants. One week after the pollinations were made the plants were re-examined and the crosses recorded as sterile or fertile as the case might be. When any two plants were found to be cross-sterile with each other on repeated trials, they were known to belong to the same intra-sterile class. Cross-pollinations of these plants

with all the other plants were then made to find out if any more plants belonged to this class. In this way the class division of the entire family was worked out. Among about forty families studied in the present investigation, not one has failed to show the division into intra-sterile, inter-fertile classes first reported by EAST and PARK (1917). While there has been an occasional sterile mating between members of different classes, this is logically to be expected and will be discussed below. Among the hundreds of plants studied, however, there has not been a single case in which a plant has been perfectly and repeatedly fertile with certain members of a class and repeatedly sterile with certain other members of the same class.

TABLE 1

Cross-pollinations within family AB. (Explanation in text).

PLANT NUMBER		CLASS l	CLASS f	CLASS g	CLASS i
	Class l				
2		0–3	4–0	1–0	0–0
7		0–5	2–2	1–0	1–0
15		0–2	8–0	2–0	1–0
	Class f				
4		3–0	2–4	1–0	1–0
10		1–2	1–10	0–0	1–0
13		3–0	0–8	1–0	1–0
16		1–1	0–5	1–0	1–0
18		3–0	0–8	1–0	1–1
19		2–0	1–7	1–0	0–0
	Class g				
9		3–0	7–0	0–0	1–0
	Class i				
14		1–0	6–1	1–1	0–0

The same cross-sterility relationships that had been observed in other self-sterile species of Nicotiana were found to obtain in *N. Forgetiana*. The 13 plants which came into flower during the time that family AB was studied, fell into five inter-fertile, intra-sterile classes. One of these classes, as has already been explained, contained 2 *N. Forgetiana* × *N. alata* hybrids. Table 1 summarizes the results of cross-pollinations with the 4 *N. Forgetiana* classes. The number of sterile and fertile combinations with each class is given for each individual. The first number in each case represents the fertile matings, the second represents the sterile ones.

Plant No. 2, for instance, was in no case fertile with other members of class l and was sterile in 3 crosses with them. It was fertile with members of class f four times, with members of class g once, and was not crossed with class i. It will be seen that only two of the thirty crosses between members of the same class were fertile. In one of these only a very small capsule was produced. It might seem from the table that there were four instead of two fertile crosses. This is because each pollination is entered twice in the table, once from the male side and once from the female. The cross-pollinations made with members of family AB *outside* the family will be discussed later. Suffice it to say that they upset all expectations by showing these four classes to be distinct from any with which they were crossed, either the classes of *N. alata* or those of the [*N. alata* × *N. Forgetiana* hybrids.

Family AD(7−AB × 18−AB)

For several reasons *N. Forgetiana* was not as satisfactory to work with as the hybrids between *N. alata* and *N. Forgetiana*. The flowers were smaller and not as easy to emasculate. In a problem involving thousands of controlled pollinations this is a serious hindrance. Furthermore, while plants of this species could be brought into bloom a second and third time, they did not, like the hybrids, regain anything like their former vigor. For these reasons little work was done with *N. Forgetiana*. A second generation was grown, however, from a cross between plant number 7 of class l and plant number 18 of class f. Ninety-four plants were grown, but a number of them died or became diseased before they were classified. The results on the remaining 86, known as family AD, are summarized in table 2. The plants fell into two classes of 41 and 45 plants.

TABLE 2

Summary of cross-pollinations within family AD (7−AB × 18−AB).

	NUMBER OF TIMES STERILE		NUMBER OF TIMES FERTILE	
	With class l	With class f	With class l	With class f
Class l	150	14	6	134
Class f	14	164	134	4

Results of cross-pollinations between families AD and AB

At the time when cross-pollinations were made between the two families, the parent plants of family AB were well along in their third blooming

period and were not at all vigorous. Whereas, during the first blooming period they had all been well over one meter high, there were now few that approached the half-meter mark and most of them had short, unbranched flower-stalks with only a few flowers. A preliminary test showed a high degree of pseudo-fertility. It might have been expected, therefore, that the back-crosses would give a high degree of pseudo-fertility. The results are shown in table 3.

TABLE 3

Cross-pollinations between family AB and family AD.

Number of sterile matings

	CLASS l–AB	CLASS f–AB	CLASS g–AB
Class l–AD	3	0	0
Class f–AD	0	10	0
Number of fertile matings			
Class l–AD	5	10	6
Class f–AD	6	5	5

There is certainly a high degree of pseudo-fertility but there are no sterile crosses between members of different classes. A few of these plants were kept until the next blooming period. Preliminary tests showed a comparatively low degree of pseudo-fertility. Out of 10 self-pollinations only 1 set seed, and that resulted in a small capsule. Cross-pollinations between these few plants yielded the results shown in table 4.

TABLE 4

Additional cross-pollinations between families AB and AD.

	CLASS l–AB	CLASS f–AB
Number of sterile matings		
Class l–AD	4	0
Class f–AD	0	5
Number of fertile matings		
Class l–AD	0	4
Class f–AD	3	0

By themselves, these results do not give conclusive proof of the identity of classes l and f of family AB with those of family AD. The more numerous data on similar crosses are all in accord with such an interpretation.

Nicotiana alata Lk. and Otto var. grandiflora Comes

Very little was done with this species, as it was even less satisfactory than *N. Forgetiana*. When grown in pots it has very few flowers and it is not possible to make as large a number of cross-pollinations as is desirable. This condition is aggravated by the fact that because of the extremely long flowers the pistils very often become injured in bagging and unbagging the inflorescence. Furthermore, this species is much more susceptible to leaf mosaic, rendering it difficult to keep parent plants for back-crosses.

Twenty-three plants, designated as family AC, were grown from an open-pollinated capsule of *N. alata*. Not all of them came into flower and it was possible to classify only 18. As shown in table 5, they fall into 3 inter-fertile, intra-sterile classes.

TABLE 5

Cross-pollinations within family AC (open-pollinated N. alata).

	CLASS o	CLASS m	CLASS p
Class o (5 plants)	0–16	19–0	13–0
Class m (6 plants)	19–0	0–14	14–1
Class p (6 plants)	13–1	14–1	2–12

In this table the first figure represents the number of fertile matings, the second the number of sterile matings. The plants of class o, for instance, have sixteen times been sterile with each other and have never been fertile. The 19 matings between classes m and o have all been fertile. The 14 between o and p have been fertile with one exception.

To summarize the results obtained within the two species:

1. The mechanism of cross-sterility in *N. alata* and *N. Forgetiana* is the same as that previously reported for their hybrids.
2. Four inter-fertile, intra-sterile classes were found in 11 plants grown from an open-pollinated seed capsule of *N. Forgetiana*. A cross between two of these classes gave a family the plants of which belonged half to one of these classes and half to the other.
3. Three classes were found in 19 plants grown from an open-pollinated capsule of *N. alata*.

Nicotiana Forgetiana × *N. alata*

EAST and PARK (1917) studied the progeny of two crosses between *N. alata* and *N. Forgetiana*. Most of the present investigation is concerned with descendants of these two crosses. Diagram 1 shows the relationships

between the various "families."[4] Each family is inclosed in a bracket. The capital letter above the bracket is the "short-hand" name of the family and agrees with those used in the earlier papers of this series.

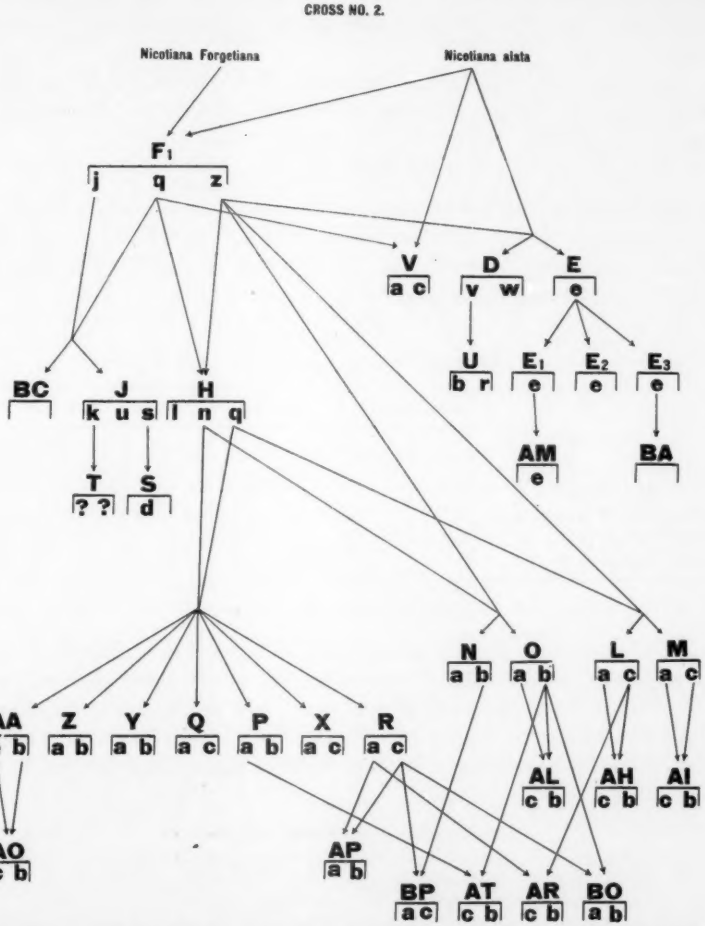

DIAGRAM 1.—The pedigree of the families of cross No. 2 discussed in this paper. The large letters designate the families, and the small letters are the symbols of the included inter-fertile, intra-sterile classes.

[4] In the following discussion "family" means a number of plants resulting from a single cross-pollination or self-pollination.

The small letters beneath the bracket are the symbols for the various inter-fertile, intra-sterile classes. Whenever two or more classes are known to be identical in their sterility and fertility relationships they are given the same symbol. To avoid confusion through duplication the class names have been changed and do not agree with those used in the earlier papers.

The results of cross-pollinations within the families will be considered first, deferring the consideration of crosses between the families, in so far as it is possible.

Family L(H−32 × F_1−44)

This family resulted from a cross between a plant in family H and one of its parents. It was found to consist of two classes, a and c, containing 18 and 24 plants, respectively. The results of cross-pollinations with this family are summarized in table 6.

TABLE 6

Summary of cross-pollinations in family L.

	WITHIN FAMILY L		OUTSIDE FAMILY L			
	Class a	Class c	Class a	Class b	Class c	Class d
Sterile with class a....	76	3	14	0	2	0
Fertile with class a....	8	52	0	7	6	1
Sterile with class c.....	3	62	2	1	11	0
Fertile with class c....	52	0	5	6	0	0

Family M (H−317 × F_1−44)

This family arose from a cross similar to that which produced family L. It originated from a cross between a plant in the same class of family H back-crossed to the same parent plant. It was found to contain the same two classes as family L. There were 21 individuals belonging to class a and 22 belonging to class c. Table 7 summarizes the results:

TABLE 7

Summary of cross-pollinations with family M.

	WITHIN FAMILY M		OUTSIDE FAMILY M					
	Class a	Class c	Class a	Class b	Class c	Class d	Class x	Class y
Sterile with class a...	60	4	11	0	1	11	0	0
Fertile with class a...	0	63	0	4	11	4	1	0
Sterile with class c...	4	78	3	0	15	0	0	0
Fertile with class c...	63	2	11	3	0	2	1	1

Family N (H−373 × F_1−44)

Families N and O originated from back-crosses with two plants of another class of family H, and the same parent plant used in the two previous back-crosses. Family N was found to consist of two classes, a and b. There were 10 plants in class a and 7 plants in class b. Table 8 summarizes the results.

TABLE 8

Summary of cross-pollinations with family N.

	WITHIN FAMILY N		OUTSIDE FAMILY N				
	Class a	Class b	Class a	Class b	Class c	Class d	Class y
Sterile with class a...	22	2	8	0	1	0	0
Fertile with class a...	2	16	0	3	4	1	3
Sterile with class b...	2	8	0	5	0	0	0
Fertile with class b...	16	0	8	0	5	1	0

Family O (H−335 × F_1−44)

Family O was found to consist of these same two classes. There were 22 plants belonging to class a and 21 plants belonging to class b. Table 9 summarizes the results.

TABLE 9

Summary of cross-pollinations with family O.

	WITHIN FAMILY O		OUTSIDE FAMILY O						
	a	b	a	b	c	d	r	x	y
Sterile with class a.....	68	3	19	0	0	0	0	0	0
Fertile with class a.....	0	68	0	9	15	1	0	3	1
Sterile with class b.....	3	42	4	10	1	0	0	0	0
Fertile with class b.....	68	2	14	0	15	6	4	2	2

Family P(H−36 × H−354)

Families P, Q, R, X, Y, Z and AA were the results of seven different crosses between various members of the same two classes of family H that had been back-crossed to produce families L, M, N and O. Family P was found to consist of 28 plants of class a and 17 plants of class b. The results are summarized in table 10.

TABLE 10

Summary of cross-pollinations with family P.

	WITHIN FAMILY P		OUTSIDE FAMILY P					
	a	b	a	b	c	d	r	x
Sterile with class a.....	110	7	11	1	2	0	0	0
Fertile with class a.....	0	61	0	1	11	0	0	1
Sterile with class b.....	7	14	1	6	0	0	0	0
Fertile with class b.....	61	0	16	1	10	3	1	2

Family Q(H−324 × H−367)

Family Q was found to consist of 13 plants of class a and 21 plants of class c. The results are summarized in table 11.

TABLE 11

Summary of cross-pollinations with family Q.

	WITHIN FAMILY Q		OUTSIDE FAMILY Q				
	a	c	a	b	c	d	x
Sterile with class a...	14	4	9	1	0	0	0
Fertile with class a...	2	29	0	5	4	1	0
Sterile with class c...	4	92	0	0	16	1	0
Fertile with class c...	29	0	16	7	0	1	3

Family R(H−362 × H−381)

Family R contained 17 plants of class a and 20 plants of class c. The results are summarized in table 12.

TABLE 12

Summary of cross-pollinations with family R.

	WITHIN FAMILY R		OUTSIDE FAMILY R					
	a	c	a	b	c	d	x	y
Sterile with class a.....	36	2	9	0	0	0	0	0
Fertile with class a.....	2	42	1	17	11	1	0	0
Sterile with class c.....	2	44	0	0	16	0	0	0
Fertile with class c.....	42	0	17	10	0	1	3	1

Family X(H−381 × H−321)

Family X consisted of 10 plants of class a and 5 plants of class c. The results are summarized in table 13.

TABLE 13
Summary of cross-pollinations with family X.

	WITHIN FAMILY X		OUTSIDE FAMILY X			
	a	c	a	b	c	y
Sterile with class a.....	22	0	4	0	0	0
Fertile with class a....	2	3	0	7	5	1
Sterile with class c.....	0	0	0	0	7	0
Fertile with class c.....	3	0	4	4	0	0

Family Y(H−382 × H−354)

Family Y consisted of 7 plants of class a and 9 plants of class b. The results are summarized in table 14.

TABLE 14
Summary of cross-pollinations with family Y.

	WITHIN FAMILY Y		OUTSIDE FAMILY Y				
	a	b	a	b	c	x	y
Sterile with class a...	8	1	5	0	0	0	0
Fertile with class a...	0	8	0	4	4	1	1
Sterile with class b...	1	20	0	5	0	0	0
Fertile with class b...	8	0	9	2	3	0	0

Family Z(H−354 × H−373)

Family Z consisted of 5 plants of class a and 9 plants of class b. The results are summarized in table 15.

TABLE 15
Summary of cross-pollinations with family Z.

	WITHIN FAMILY Z		OUTSIDE FAMILY Z			
	a	b	a	b	c	x
Sterile with class a.....	12	0	6	0	0	0
Fertile with class a....	0	9	0	2	2	0
Sterile with class b.....	0	16	0	3	0	0
Fertile with class b....	9	0	8	0	2	3

Family AA(H−381 × H−322)

Family AA consisted of 6 plants of class a and 10 plants of class c. The results are summarized in table 16.

TABLE 16

Summary of cross-pollinations with family AA.

	WITHIN FAMILY AA		OUTSIDE FAMILY AA				
	a	c	a	b	c	d	r
Sterile with class a...	18	0	5	0	0	0	0
Fertile with class a...	0	9	1	6	4	0	1
Sterile with class c...	0	8	0	0	11	0	0
Fertile with class c...	9	0	2	2	0	1	0

Family W(F_6 of cross No. 1)

This family was a sixth-generation descendant of cross No. 1. It was found to consist of two classes, x and y, containing 13 and 18 plants, respectively. Table 17 summarizes the results.

TABLE 17

Summary of cross-pollinations with family W.

	WITHIN FAMILY W		OUTSIDE FAMILY W			
	x	y	a	b	c	d
Sterile with class x.....	22	2	0	0	0	0
Fertile with class x....	0	7	6	8	10	2
Sterile with class y.....	2	4	0	0	0	0
Fertile with class y....	7	0	8	2	3	2

Family S(J−473 selfed)

As has already been discussed by EAST and PARK (1917), it is occasionally possible to self-fertilize these self-sterile plants and obtain a small capsule of seed. Two such selfings of plants in family J were grown. One of them, family T, was discarded because the plants were diseased. The other, family S, consisted of only one class, a further corroboration of EAST's earlier work. The results with this family are summarized in table 18.

TABLE 18

Summary of cross-pollinations with family S.

	WITHIN FAMILY S	OUTSIDE FAMILY S				
	d	a	b	c	x	y
Sterile with class d.....	116	1	0	2	0	0
Fertile with class d.....	2	11	8	14	2	2

Family U(D—175 selfed)

Family U resulted from a selfing of a plant in family D. Six plants were grown. Three of them belonged to class b and three to class r.

Family V(F_1—10 × *N. alata*)

Family V resulted from back-crossing one of the first-generation plants of cross No. 2 to a plant of *N. alata*. It was in a sense similar to families D and E, which had resulted from similar back-crosses. It was found to consist of 13 plants of class a and 18 plants of class c. The results are tabulated in table 19.

TABLE 19

Summary of cross-pollinations with family V.

	WITHIN FAMILY V		OUTSIDE FAMILY V						
	a	c	a	b	c	d	r	x	y
Sterile with class a.....	14	3	9	1	0	0	0	0	0
Fertile with class a.....	0	33	0	3	5	0	1	0	1
Sterile with class c.....	3	70	5	0	13	1	0	0	0
Fertile with class c.....	33	0	13	4	0	9	1	3	0

The remainder of the families were grown in very small numbers and no more crosses were made than were necessary to establish the number of classes present and to determine their identity or non-identity with the classes already studied.

Family AH(L—18 × L—20) was found to consist of 8 individuals of class b and 9 individuals of class c.

Family AI(M—30 × M—25) consisted of 9 individuals of class b and 11 individuals of class c.

Family AL(O—32 × O—46) consisted of 8 individuals of class b and 12 of class c.

Family BO(R−2 × O−2) consisted of 4 individuals of class a and 6 of class b.

Family BP(N−7 × R−12) consisted of 6 individuals of class a and 4 of class c.

Family AO(AA−2 × AA−13) consisted of 6 individuals of class b and 4 of class c.

Family AP(R−20 × R−13) consisted of 7 individuals of class a and 9 individuals of class b.

Family AT(P−27 × O−2) consisted of 11 individuals of class b and 6 individuals of class c.

Family AS(W−1 × W−5) had resulted from a cross between the two classes of family W, a descendant of the first cross. No parent plants were available for testing the relationships of the class of family W with those of family AS. The latter was found to consist of two classes, class dd with 8 individuals, and class df with 6.

Families AY(M−21 × W−11) and AZ(X−15 × W−17) resulted from two crosses between family W and cross No. 2. Family AY consisted of 2 plants of class sa, 3 of class sb, 6 of class sc, and 1 of class sd. Family AZ consisted of 4 plants of class ra and 5 plants of class rb.

The results for all the families are summarized in table 20.

TABLE 20

Summary of all families.

NAME	ORIGIN	CLASSES PRESENT AND NUMBER OF PLANTS IN EACH
Family L	H-328 × F_1-44	18-a 24-c
Family M	H-317 × F_1-44	21-b 20-c
Family N	H-373 × F_1-44	10-a 7-b
Family O	H-335 × F_1-44	22-a 21-b
Family P	H-376 × H-354	28-a 17-b
Family Q	H-324 × H-367	13-a 21-c
Family R	H-362 × H-381	17-a 20-c
Family X	H-381 × H-321	10-a 5-c
Family Y	H-382 × H-354	7-a 9-b
Family Z	H-354 × H-373	5-a 9-b
Family AA	H-381 × H-322	6-a 10-c
Family S	J-473 selfed	41-d
Family T	J-489 selfed	? ?
Family U	D-175 selfed	3-b 3-r
Family V	F_1-10 × *N. alata*	13-a 18-c
Family W	Cross No. 1, F_3	10-x 8-y
Family AB	*N. Forgetiana*	7-f 4-l 2-g 1-i
Family AC	*N. alata*	6-o 6-p 5-m
Family AD	AB-7 × AB-18	40-l 45-f

TABLE 20 (continued)

Summary of all families.

NAME	ORIGIN	CLASSES PRESENT AND NUMBER OF PLANTS IN EACH			
Family AH	L–18 × L–20	8–b	9–c		
Family AI	M–31 × M–25	9–b	11–c		
Family AL	O–32 × O–46	8–b	12–c		
Family AO	AA–2 × AA–13	6–b	4–c		
Family AP	R–20 × R–13	7–a	9–b		
Family AR	R–38 × L–24	13–b	7–c		
Family AT	P–27 × O–2	11–b	6–c		
Family AS	W–1 × W–5	8–dd	6–df		
Family AY	M–21 × W–11	2–sa	3–sb	6–sc	1–sd
Family AZ	X–15 × W–7	4–ra	5–rb		
Family BO	R–2 × O–2	4–a	6–b		
Family BP	N–7 × R–12	6–a	4–c		

TABLE 21

Summary of cross-pollinations between classes. x=fertile, o=sterile.

a	b	c	d	e	r	l	f	g	i	m	o	p	sa	sb	sc	sd	ra	rb	ga	gb	gc	x	y	dd	df	h	
o	x	x	x	x	x	x	x		x	x		x	x		x	x	x		x	x		x	x	x	x	x	a
	o	x	x	x	x	x	x	x		x	x	x	x			x	x		x	x	x	x	x	x	x	x	b
		o	x	x	x	x	x	x	x	x		x	x		x		x		x	x		x	x	x	x	x	c
			o		x	x	x	x	x														x	x				d
				o			x				x													x	x	x		e
					o																						r
						o	x	x	x	x	x	x												x	x		x	l
							o	x	x	x	x	x												x	x	x	x	f
								o	x		x	x									x		x	x	x	x	g
									o																		i
										o	x	x												x			m
											o	x					x			x				x		x	o
												o			x									x		x	p
													o	x	x	x	x	x	x	x	x			x			sa
														o	x	x	x	x	x	x							sb
															o	x	x	x	x					x			sc
																o	x	x		x				x			sd
																	o	x	x	x	x			x	x		ra
																		o									rb
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																				o	x				x		gb
																					o						gc
																								x		x	x
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																										o	h

Cross-pollinations between the families

The same procedure was followed in establishing the relations between the classes of the different families as had been followed in working with the individual families. If crosses between two classes in different families were repeatedly sterile the two classes were known to be identical and were given the same symbol. These data are summarized in table 21. The data for each family have already been presented in tables 6 to 19.

If there had been any need for more proof of the reality of this system of inter-fertile, intra-sterile classes, it was given when all the classes a, all the classes b, and all the classes c, were crossed together. From the results obtained, the a's might all have been from one family, or the b's, or the c's. Each individual was sterile with all the other members of that class—no matter from what family—and fertile with members of all other classes. There were a few exceptions, to be sure, but they formed less than four percent of the cases, and none of them could be duplicated by repeating the same pollination. *There was not a single instance in which a plant was repeatedly sterile with certain members of a class and repeatedly fertile with certain other members of the same class.* Table 22 summarizes the results of these inter-family crosses.

DISCUSSION

Working hypothesis

The following were among the facts established by the earlier work on the subject:

1. Self-sterility is heritable.
2. Under normal conditions, the pollen-tubes produced by pollen from any self-sterile plant will not grow in the styles of that plant with a rapidity sufficient to reach the ovules during the "life" of the flower.
3. Similarly, under normal conditions, the pollen-tubes of this plant will not grow in the styles of any other plant with which it is cross-sterile with a rapidity sufficient to reach the ovules during the "life" of the flower.
4. Pollen-tubes from pollen of cross-fertile plants will grow with a rapidity sufficient to allow fertilization.

This work had suggested certain conclusions and from them the following working hypothesis was assumed in beginning the present investigation:

The phenomena of 2, 3 and 4 are due to the action of certain essential factors. When two plants—and hence the pollen-tube and the style—

TABLE 22

Results of cross-pollinations between families containing classes a, b and c. o indicates cross-sterility; x indicates cross-fertility. a–L means members of class a of family L, a–M means members of class a of family M, etc.

a–L	a–M	a–N	a–O	a–P	a–Q	a–R	a–V	a–X	a–Y	a–Z	a–AA	c–L	c–M	c–Q	c–R	c–V	c–X	c–AA	b–N	b–O	b–P	b–U	b–Y	b–Z	
o	o	o	o	o	o	o	o			o	o	x	x	x	x	x			x	x	x			x	a–L
	o	o	o	o	o	o	o				o	x	x	x	x	x			x	x	x	x			a–M
		o	o	o	o	o	o				o	x	x	x	x	x			x	x	x				a–N
			o	q	o	o	o	o	o			x	x	x	x	x				x	x	x	x		a–O
				o	o		o	o				x	x	x	x	x				x	x			x	a–P
					o	o	o				o	x	x	x	x	x				x	x			x	a–Q
						o	o			o	o	x	x	x	x	x		x	x	x	x			x	a–P
							o		o	o	o		x	x	x	x	x	x	x					x	a–V
								o	o				x	x	x		x			x	x				a–X
									o	o				x	x					x	x			x	a–Y
										o				x		x			x		x			x	a–Z
											o	x	x	x	x	x		x	x		x		x	x	a–A
												o	o	o	o	o	o	o	x	x				x	c–L
													o	o	o	o	o	o	x	x	x				c–M
														o	o	o	o	o	x		x	x	x		c–Q
															o	o	o	o	x	x	x			x	c–R
																o	o	o	x	x	x	x			c–V
																	o	o		x					c–X
																		o	x		x		x		c–A
																			o	o	o	o	o	o	b–N
																				o	o	o	o	o	b–O
																					o		o	o	b–P
																						o	o		b–U
																							o	o	b–Y
																								o	b–Z

differ for these factors, a stimulus is produced which causes the accelerated growth of cross-fertile matings. When, on the other hand, as in 2 and 3, the pollen-tube and the style have the same essential factors in common, there is no stimulus and no accelerated growth. These essential factors are heritable and given suitable material, it should be possible to demonstrate their presence and the manner of their inheritance.

Number of classes

As little had previously been done in identifying the classes of different families with one another, a number of questions immediately presented themselves:

In these crosses between *N. alata* and *N. Forgetiana* are we dealing with a few classes or with a great many? Would we obtain these same classes upon making further crosses between the two species or would we obtain different ones? Is the mechanism of cross-sterility the same in the pure species as in the hybrids? Can we find any of these same classes (and hence the same factors) in *N. alata* and *N. Forgetiana?* How are these classes passed on from generation to generation?

Some of these questions can now be answered definitely: In the first place, there are many more classes than had at first been suspected. In the more or less inbred descendants of cross No. 2 between *N. alata* and *N. Forgetiana* there are at least five and perhaps six different classes, the relationships between two of them never having been determined. In the sixth generation descendants of the first cross (family W) there were two classes which were distinct from the five classes of the second cross with which they were tried. A seventh generation was grown from these plants (family AS) and was found to contain two classes. It was not possible to test them with the parent plants of family W. They were, however, different from any of the classes with which they were crossed. There were at least four classes in the few *N. Forgetiana* plants grown, and three classes in the *N. alata* plants. These seven classes were distinct from each other and from all the other classes with which they were tested. In crosses between family W and cross No. 2, seven classes were obtained. They were all different from each other, from the parent classes, and from all other classes with which tests were made.

All of these data are summarized in table 22 where the inter-fertility relationships of these 27 classes are indicated. We have good reason to believe that the two classes of family AS, dd and df, may be the same as the two parent classes, x and y, of family W. It is also quite possible that class r and class e are identical. All other cases in which nearly related

families might have been expected to contain identical classes have been adequately tested. This leaves 24 classes. In so far as they have been tested, the 24 are all distinct from one another and there is little reason for believing that each one is not distinct from every other one.

In the present investigation we have grown a few more or less inbred descendants of two crosses between *N. alata* and *N. Forgetiana;* two crosses between these two strains; a few plants from an open-pollinated capsule of *N. Forgetiana;* and a few plants from an open-pollinated capsule of *N. alata.* In these few samples 24 classes have been found. It seems probable that in the two species and in the various combinations between them, there must be a great many more possible classes. If this be true it is not at all surprising that the two crosses made by EAST and PARK (1917) differed considerably as regards their class behavior.

Hereditary nature of classes

There was every indication that these classes have a genetic basis. This phase of the work is being continued and for the present the evidence may be summarized as follows:

1. Nearly related families possessed certain classes in common, while the classes of more distantly related families were all distinct from one another.
2. The same classes appeared in successive generations.
3. While the details are yet to be worked out, the classes are apparently inherited in regular Mendelian fashion.

These points are demonstrated in diagram 1. There it will be seen that all of the more or less inbred descendants of family H consisted of only three classes and that there was a certain regularity in the manner of their inheritance. Families U, V and E, less nearly related, contain some of these same classes and certain others which are distinct. The classes in cross No. 2 are distinct from those in cross No. 1, as are also the classes in *N. alata* and *N. Forgetiana.*

SUMMARY

1. The same system of inter-fertile, intra-sterile classes was found to exist in *N. Forgetiana* and in *N. alata* as had previously been found to occur in their hybrids and in other self-sterile species of Nicotiana.

2. Among the few strains of *N. Forgetiana, N. alata,* and *N. alata* × *N. Forgetiana* that were studied in the present investigation, there were at least 24 such classes. There must be many more in the two species as a whole. From one open-pollinated capsule of *N. Forgetiana* 4 classes were

TABLE 23

Tabular comparison with other work on self-sterility.

MATERIAL	REPORTED BY	CROSS-STERILITY	INTER-STERILE CLASSES	PSEUDO-FERTILITY	CLASSES INHERITED	STERILITY RECESSIVE IN CROSSES WITH SELF-FERTILE SPECIES
Nicotiana alata	ANDERSON (this paper)	——	——	——	——	——
N. Forgetiana	ANDERSON (this paper)	——	——	——	——	——
N. alata × *N. Forgetiana*	EAST and PARK (1917); ANDERSON (this paper)	——	——	——	——	
N. commutata	EAST and PARK (1917)	——		——		
N. commutata × *N. Forgetiana*	EAST and PARK (1917)	——	——	——		
N. angustifolia	EAST and PARK (1917)	——	——			
N. glutinosa	EAST and PARK (1917)	——		——		
Linaria vulgaris	DEVRIES (1906); CORRENS (1912–1916)	——	——	——		
Antirrhinum hispanicum	BAUR (1919)	——	——	——		——
Veronica syriaca	LEHMANN (1919)	——	——	——		
Cardamine pratensis	CORRENS (1912)	——	?	——	?	
Reseda odorata	COMPTON (1912)					——
Bursa grandiflora	SHULL (1923)	——	——	——		

obtained; from a similar capsule of *N. alata* 3 classes were obtained. These 7 classes were all distinct from each other and from classes in the hybrids.

3. These phenomena are interpreted as due to the action of certain factors. Plants which are cross-sterile with each other (which belong to the same class) have certain essential factors in common. Plants which are cross-fertile with each other (which belong to different classes) differ for these factors.

4. These factors are germinal and are passed down from generation to generation. Two lines of evidence point to this interpretation: a. The common possession of certain classes by nearly related families and the absence of any classes in common by families less closely related. b. The regularity with which these classes appear in subsequent generations.

This, then, is the status of the self-sterility problem in Nicotiana. Table 23 shows how certain of these results have been confirmed by the work of other investigators. This close corroboration justifies the suggestion that the conditions reported here may be somewhat general among self-sterile plants.

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INHERITANCE OF EGG SIZE IN *DROSOPHILA MELANOGASTER*

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INTRODUCTION

It has been demonstrated in a few cases that differences in size and height are inherited in the same way as are other Mendelian characters, but in most cases it has been found that size differences cannot be explained upon such a simple basis. Such cases are generally referred to multiple factor differences, but it has rarely been possible to demonstrate the presence of these postulated factors. At most the inference is made probable by reference to the spread of the F_2 as compared with the F_1 generation. Drosophila is the only form in which all of the linkage groups are so well known that it is possible to locate definitely any factors that determine or modify the size of particular parts or organs. The egg of Drosophila is especially favorable material for such genetic study, not only because here any postulated factors can be traced, but also because the size of the egg has been found to be independent of the effects of the environment and even of the size and age of the female producing the egg.

The eggs of various mutant stocks of Drosophila exhibit characteristic

differences in size. Whether this variation in size is one of the effects of the gene that also conditions the mutant character, or whether it is the result of other genes present in the stocks could only be learned by crossing.

The size of the egg might be determined either by its own genetic composition or (through the egg membranes) by the germinal constitution of the female that produces it. Since the egg before maturation and the tissue cells of the female have identical genetic factors, it is not possible to discover by genetic methods whether the egg or the parent tissues determine the size of the egg. However the size of the egg is determined, the inheritance is not strictly maternal (although the term has sometimes been used in this sense) because egg size is as characteristic of the female as is any somatic character.

The size of the egg is fixed before the entrance of the sperm and can not possibly be changed by the genes from the male. The fertilized egg, that develops into the F_1 embryo, should have the size characteristic of the P_1 maternal race and the egg hatching into the F_2 embryo will be expected to show first the effects of the genes brought in by the P_1 sperm. The size of F_1 eggs (hatching into F_1 embryos) is therefore characteristic of the P_1 female, and the size of the F_2 eggs is characteristic of the F_1 female.

METHODS

The females, whose eggs were to be measured, were isolated in one-fourth pint milk-bottles. The eggs were laid on ripe banana, that had fermented in a yeast solution for at least twenty-four hours. To facilitate the removal of the eggs from the bottles, the food was placed between layers of paper toweling on an ordinary microscopic slide. Four thicknesses of the toweling were used and a small piece of the fermented banana, the size of a grain of corn, was placed between the two uppermost layers. The strips of toweling were then thoroughly saturated with the fermented banana juice. If too small an amount of the juice is used the food becomes dry, if too great the juice is likely to run off into the bottle, and drown the flies. Food prepared in this way should keep moist for twenty-four hours. Food that has fermented for more than four or five days becomes too acid. The object of placing the banana between the layers of toweling is to prevent the flies from burying their eggs in the banana. Eggs thus buried are likely to have portions of the banana clinging to them and are difficult to measure accurately. Eggs laid on the moist paper may be removed in a clean condition. Covering the banana with the single layer of toweling in no way reduces egg laying.

In order to avoid loss from hatching, it was necessary to measure the

eggs at intervals of about twenty hours. All stocks and isolated females were kept in an incubator where the temperature was approximately 25°C. For measurement the eggs were removed from the food with a dissecting needle, the point of which had been flattened into a spatula-like form and then ground to an oblique, blade-like edge. Working under a binocular, the eggs could be rapidly scooped up with the needle and removed to a slide where they were placed in rows in a film of water for measurement under the compound microscope. For measurement, the low power objective and a No. 2 Leitz micrometer eye-piece were used.

DESCRIPTION OF THE EGG

The egg of *Drosophila melanogaster* averages about .5 millimeter in length. It is white in color and its chorion shows hexagonal sculpturing due to the flattened follicle cells surrounding it. Near one end of the egg, which corresponds to the anterior end of the embryo, is found a pair

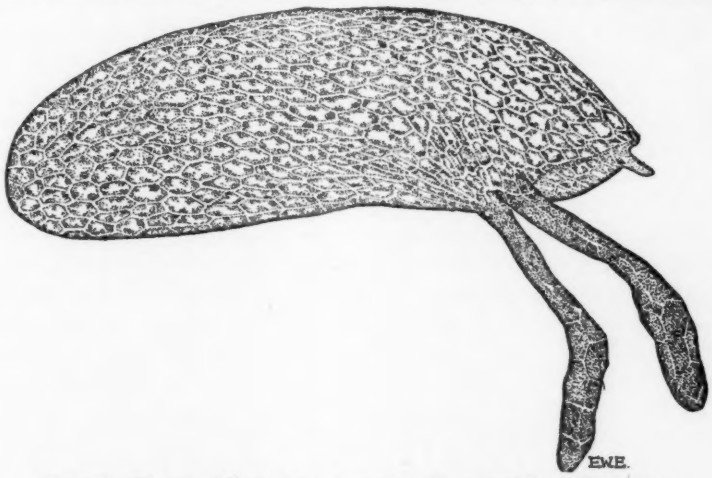

FIGURE 1.—The egg of *Drosophila melanogaster*. Drawn by Miss E. W. EMMART.

of slender filaments. The function of these appendages is unknown, but it has been suggested that they may aid in keeping the egg afloat. The micropyle cone is found at the anterior end of the egg. Figure 1 shows the egg in position for measuring.

VARIATION IN DIFFERENT MUTANT STOCKS

Most of the stocks examined for egg size were being kept for some one or more mutant characters. Some of these stocks had been inbred for a

long time. Wild stocks which had recently been brought into the laboratory and which carried no observed mutation were also measured. Over a hundred stocks were examined. In determining the range of egg size, a minimum of one-hundred eggs were measured from each stock, and, in cases where the stocks were extensively used in crosses, as many as a thousand eggs were measured. Several pairs of flies were isolated so that eggs from a number of females would be included in the measurements.

Table 1 shows the range of 26 stocks taken at random. The numbers given at the head of the columns, ranging from 22 to 31 divisions or units, represent the length of the egg in units of the eye-piece micrometer. A unit equals .018 mm. The name of the stock usually indicates the single

TABLE 1

Size range of various stocks.

STOCKS	UNITS AND FREQUENCIES									
	22	23	24	25	26	27	28	29	30	31
Apricot			2	5	8	20	34	23	7	1
Florida wild				2	9	33	43	11	1	1
X ple		1	2	8	25	32	25	5	2	
Abrupt						2	29	43	23	3
Purple			1	22	37	33	6	1		
w_a *v f*					2	10	38	36	13	1
Bar	3	6	23	51	15	2				
Dichaete				11	27	40	19	3		
Lobe				11	48	29	8	4		
Aristaless				4	11	29	37	18	1	
Morula					1	12	30	42	15	
Facet				1	3	17	47	26	6	
Oblique					3	29	40	24	4	
Cream					3	23	35	28	11	
Eyeless			1	5	10	34	31	14	5	
Wild Ku		2	8	45	19	25	1			
s_8 *ab cv* c_{ps} *l v*				12	36	38	12	2		
7 ple		1	3	5	12	29	28	19	3	
Curly				6	44	40	9	1		
Shaven				2	15	30	23	20	9	1
Cardinal			4	23	43	25	4	1		
Ruby	1	3	14	33	34	14	1			
Ivory			4	34	32	24	6			
Vestigial	2	8	24	22	20	18	4	2		
Sooty				2	9	23	32	16	17	1
White					1	11	38	40	10	

It is seen that there is considerable individual variation in the egg lengths in these twenty-six stocks. The ranges determined are fairly constant for the different stocks.

mutant character of that stock. The different stocks show much individual variation. Records made at different periods indicate that the range for a stock is fairly constant. The most extreme stocks have quite different means. The means of others range between these extremes. No one condition can be designated as the normal or wild type. The stock listed as Florida wild had been in the laboratory only one generation when the record was made.

In studying the genetic behavior of the size variations, the two most divergent stocks were most extensively used. These are abrupt, which has a mean of 29.0 units (from the measurement of one-thousand eggs), and bar, which has a mean of 24.7 units. There is very little overlapping of the extremes of these two stocks. Apricot-vermilion-forked and apricot were also used extensively. Ruby was used in several crosses.

CONSTANCY OF THE CHARACTER

One of the first steps was to determine whether egg size is dependent upon environmental conditions. It is well known that the adult vinegar fly varies greatly in size, depending upon food conditions. In order to determine the relation of the size of the eggs to that of the female which lays them, a virgin female and a single male were placed in a bottle containing scarcely enough food to carry through an average brood. The first females to emerge were large, while the last ones were much smaller. The eggs of the large females were compared with those of the small ones. Since wing length has been found to be a fairly reliable index of the size of flies, this was used as a measure. The wing length given is the distance between the anterior cross-vein and the tip of longitudinal vein III. Abrupt females and hybrid females from the cross of abrupt by bar were used. Since the heterozygous condition of a character has sometimes been shown to be more susceptible to environmental influences than the homozygous condition, flies of both constitutions were tested (see table 2). The

TABLE 2

Effect of size of female upon egg size.

	ABRUPT 1*		ABRUPT 4*		F_1 (ABRUPT × BAR) 1*		F_1 (ABRUPT × BAR) 4*	
Mean wing length....	66.0	86.4	64.8	85.4	69.6	91.2	68.4	91.2
Mean egg length.....	28.9	28.7	29.1	29.5	26.9	27.0	27.5	27.0

* These numbers are culture numbers.

The mean length of eggs of sister flies which differ in size. Eggs laid by small females average as long as those of large females.

means for egg length were computed from the measurements of two-hundred eggs in each case. The results show that neither in the homozygous nor in the heterozygous condition is there any correlation between the size of the female and the size of the egg she lays. Small females lay very few eggs, but the eggs laid have an average as large as that of larger females.

As a culture ages the bottle becomes dryer. Consequently the first flies to emerge develop under conditions of much greater moisture than do those which are last to emerge. Since the size of the egg did not change, we may conclude that moisture has no effect upon egg size.

The relation of the size of eggs to the age of the female producing them was also examined. Eggs were measured at different periods in the life of the same female. The means were computed for the eggs in lots of fifty each. Records were made for two females from the abrupt stock and from one wild-stock female (Wild Ku). Table 3 gives the data. The age

TABLE 3

Effect of age of female upon egg size.

WILD KU										
Age (days).....	2	5	7	34	38	41	43	46	51	59
Mean egg length...	26.3	24.9	25.7	26.1	26.5	26.9	26.6	26.0	26.4	26.7
ABRUPT 2										
Age (days).....	2	7	10	12	14	18				
Mean egg length...	28.9	29.0	28.9	28.6	28.7	28.5				
ABRUPT 4										
Age (days).....	2	7	9	11	13	16				
Mean egg length...	29.1	28.9	28.9	28.4	28.3	28.4				

This table shows that there is little, if any, change in the length of the egg, which is correlated with the age of the female laying it.

of the female at the beginning of each lot is given. In the case of the two abrupt females there is a slight decrease in size with age. This decrease, however, is very small. In the case of the wild female there is no indication of any decrease in the size of the egg of older flies.

Another environmental condition, temperature, was also examined. Three temperature conditions were utilized,—one ranging from 28° to 30°C, another from 23° to 26°C, and a third from 10° to 20°C. In order to

have all conditions (excepting temperature) as nearly identical as possible, the daily egg production of a single female (previously mated to a single male) was divided among the three temperature conditions. Females reared from the eggs of a single female but subjected during development to different temperature conditions, were compared as to egg size. Two abrupt females were used in this experiment. Table 4 gives the mean lengths of two-hundred eggs. The egg size under all these conditions was found to be constant.

TABLE 4

Effect of temperature upon egg size.

	INCUBATOR 23° to 26°C	INCUBATOR 28° to 31°C	COLD 10° to 20°C
Abrupt 4.....	28.8	29.3	28.8
Abrupt 8.....	28.8	28.6	28.5

The mean egg-lengths for flies reared under different temperatures indicate that temperature does not influence egg-size.

In spite of what has just been said with reference to egg size, there was evidence that some conditions do, to a slight extent, influence egg size; for individual females showed some variation from day to day. What the factors are that cause such fluctuations was not determined. Nevertheless, the constancy of egg size in general makes it an excellent character for a study of genetic behavior.

STOCKS USED IN CROSSES

The two stocks most used in crosses were abrupt and bar. The mean of abrupt is 29.0 and that of bar 24.7. Figure 2 shows the range in egg size in these two stocks. There is practically no overlapping of the extremes. Figure 3 shows the range of individual means for a number of females from each stock. Each point of the graph represents the mean of fifty eggs from a single female. While there is some individual variation, the difference between the nearest extremes of the two stocks is distinct. Another stock, apricot-vermilion-forked, was used to some extent. It had been kept for about a year as a stock combining these three sex-linked characters. The mean of apricot-vermilion-forked, computed from five-hundred egg measurements, was 28.5. Table 1 shows that this stock also has a long egg but not as long as the abrupt stock.

Ruby has a short egg with a mean of 25.4 (computed from five-hundred eggs). It is, however, not as short as bar.

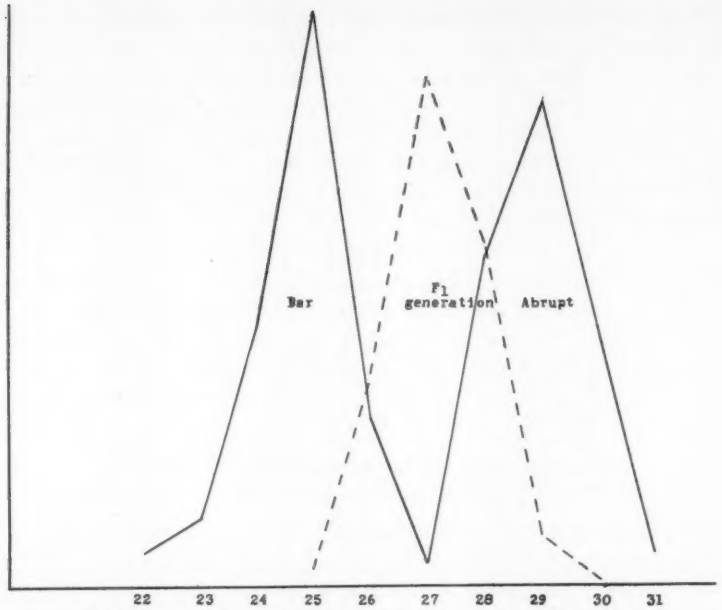

FIGURE 2.—Curves for egg length of bar and abrupt, together with that of the F_1 generation from crossing the two stocks.

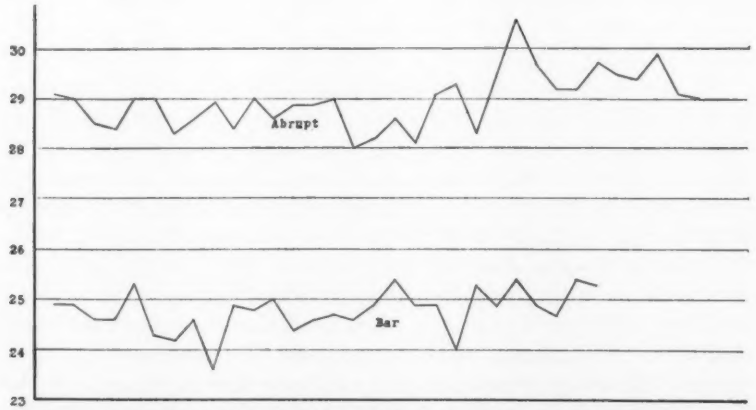

FIGURE 3.—Showing individual variation of females from the bar and abrupt stocks. Each point on the graph is an individual mean from fifty eggs. There is some individual variation but the two stocks behave quite differently.

EGG SIZE AND MUTANT CHARACTER

The following experiments were made to determine whether the genes that condition egg size are identical with those which produce the mutant characters of the two stocks abrupt and bar. The curves for the range of abrupt (long) and for bar (short) are shown in figure 2. The curve for egg measurements from F_1 flies is also included. It is seen that the curve for eggs from F_1 flies falls almost exactly intermediate between the two stocks. The crosses were made in both directions, but no significant difference was found between the reciprocal crosses (table 5).

TABLE 5

Comparison of range of reciprocal crosses of bar by abrupt.

UNITS	25	26	27	28	29	30	31	MEAN
Bar female by abrupt male	6	80	208	100	6			27.1
Abrupt female by bar male	6	65	152	142	29	6		27.4

There is no significant difference in egg-length between reciprocal crosses of bar and abrupt.

From figure 4 it is seen that the egg size is independent of the mutant character of the stock. This figure shows the results of the F_2 generation from the cross between bar female and abrupt male. Due to the amount of labor involved in determining the genetic constitution of the eggs of any one female (the measurement of at least fifty eggs) the number of individuals considered is small. Fifty eggs were measured from each of nine females. All of these females were F_2 segregated bars. Although a very few individuals were considered, both parental extremes were realized. That the egg size is independent of the mutant character is evident from the fact that some of the bar females show an egg size identical with that of the abrupt stock. There was segregation of the long egg that is characteristic of the abrupt stock, but these long eggs were produced by bar females. Thus the F_2 generation shows that egg size segregates independently of the mutant characters in the stocks.

GENETIC BEHAVIOR OF LONG EGG CONDITION FOUND IN ABRUPT STOCK

An abrupt female was crossed to a bar male and the F_1 male was back-crossed to an abrupt female. This cross was made to test whether any of the factors involved in producing the long egg of the abrupt stock are located in the X chromosome. The eggs measured were produced by F_2 females from this back-cross. Figure 5 shows the origin of the X and of

the second chromosomes carried by these females. The letters inside the chromosomes indicate whether the particular chromosome came from the long or short stock. Figure 5 shows that every female derives both of her sex chromosomes from the long-egg stock (abrupt). From among the F_2 females forty were tested for egg size. Fifty eggs from each female were

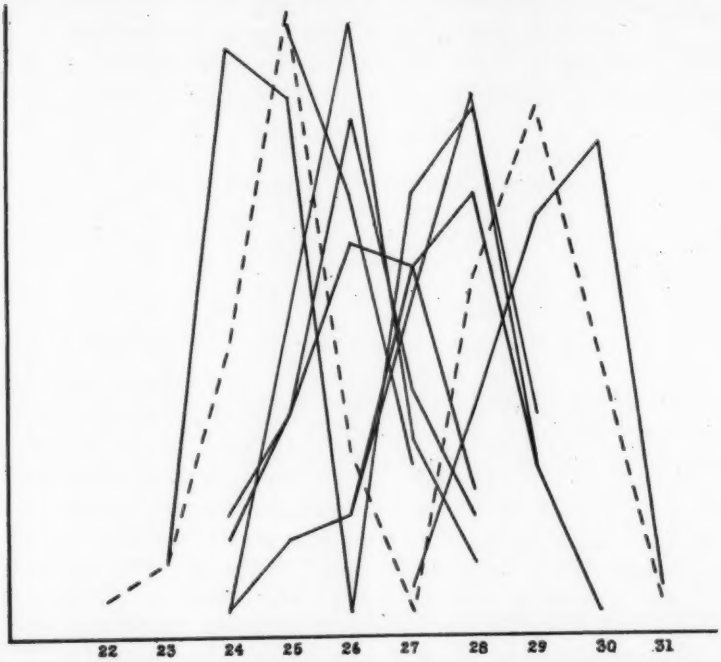

FIGURE 4.—In this graph are shown the F_2 results of the cross, bar female by abrupt male. The females tested were all segregated bars and they are indicated by the solid lines in the graph. The dash curve to the left shows the range of the bar stock and the same type of curve to the right, the range of the abrupt stock. Segregation of both parental types was obtained here.

measured and the range of the means for these females is given in figure 6. Every one, with a single exception, fell within the range of the abrupt (long) stock. The range shown by the graph in figure 6 may be compared with the range for abrupt shown in figure 3. If the long egg condition is due to a single autosomal gene we would expect only half of these F_2 females to be pure for the long condition, but all of the females show the long-egg tendency. The only explanation that will satisfy this condition

is that the factor for long egg (or at least the main one) is located in the sex chromosome.

In the graph of figure 6 two lines are shown. The dash line represents the means of females which show the abrupt character and the solid line represents the means of females which do not show the abrupt condition. Abrupt is a recessive mutation in wing venation and tests have shown that the gene for abrupt is located in the second chromosome. Females which show the segregated abrupt character are known to have received both of their second chromosomes from the abrupt (long) stock. Likewise females from this cross which do not show the abrupt character are known to have

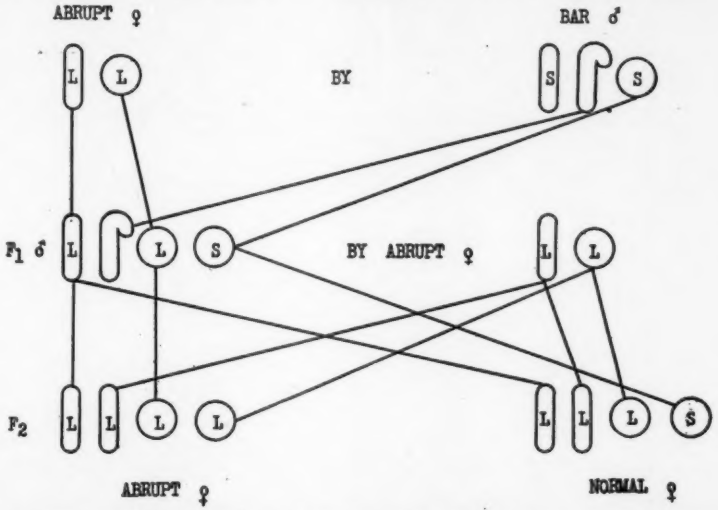

FIGURE 5.—Showing the origin of the sex chromosomes and third chromosomes in F_2 females from the cross as indicated above.

received one of their second chromosomes from the bar (short) stock. The average of the normal (non-abrupt) females is lower than that of the abrupt females. The same results are shown in figure 7. Here the dotted curve represents the range of five-hundred eggs from the pure abrupt stock. The dash curve shows the range of five-hundred eggs from ten segregated abrupt females taken at random from those shown in figure 6. The solid curve represents the range of five-hundred eggs from ten of the normal females shown in figure 6. The curve for the segregated abrupt females practically coincides with that of the abrupt stock. The mean of the curve of the normal females is somewhat less than that of the abrupt

stock. Two interpretations may be placed upon the significance of this difference between wild and abrupt females. It is possible that some minor factor for the long (abrupt) condition is located in the second chromosome, since the abrupt females which here are known to have not only both X chromosomes but also both of their second chromosomes from the long stock, produce longer eggs than normal females, which are known to have

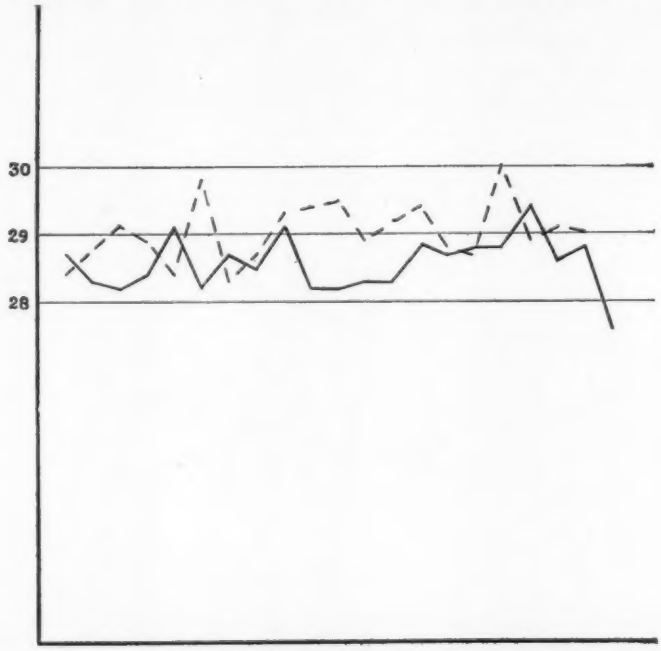

FIGURE 6.—Results of back-crossing an F_1 male from the cross, abrupt female by bar male, to an abrupt female. Each point in the graph represents an egg mean (from 50 eggs) for an F_2 female. The dash line indicates the range of segregated abrupt females, and the solid line, wild females from the same cross. The abrupt females lay eggs which average slightly larger than those of wild females, but practically all of the females examined from this cross fell within the range of pure abrupts. (See figure 3.)

but one second chromosome from the long stock. Another explanation, which further data show to be correct, is that a factor which tends to produce the short length found in the bar stock is located in the second chromosome; for, the normal females which show an average range less than pure abrupts have derived one of their second chromosomes from the bar stock. Linkage data (discussed later) indicate that both the above-

mentioned conditions may be true. That there are in the second chromosome of the bar stock one or more genes which decrease the size of the egg and one or more genes in the same chromosome of the abrupt stock which increase the length of the egg. Figure 5 shows the origin of the second chromosomes in these two types of females. From the data just considered it follows that at least one major factor for the long egg condition belongs to the sex-linked group.

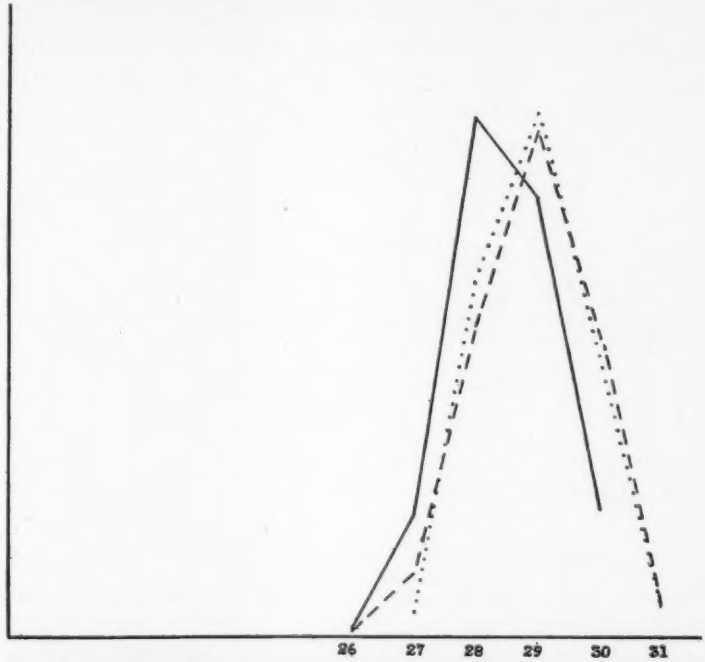

FIGURE 7.—The mass results of the data given in figure 6. The dash curve shows the range of segregated abrupt; solid curve, segregated wilds; and dotted curve, pure abrupt stock.

To further check the data just given, a back-cross was made from the reciprocal of the previous cross. An F_1 male from the cross, bar female by abrupt male, was back-crossed to an abrupt female. Eggs were measured from F_2 females from this cross. Figure 8 shows the origin of the sex chromosomes and the second chromosomes in these females.

Figure 8 shows that each female receives one member of her pair of X chromosomes from the abrupt stock and the other member from the bar stock. Figure 9 shows the range of the means of twenty-eight females

from the F_2 of this back-cross. This graph shows that the F_2 behavior here is quite different from that shown by the reciprocal cross (figure 6). The difference between these females and those of the reciprocal cross is that in the former cross they receive both their X chromosomes from the abrupt (long) stock and here one of the two X chromosomes comes from the bar (short) stock. Only about half of the females have a mean which falls within the range of the pure abrupt (long), the rest falling below. Other data have indicated that one of the main factors for long egg is in the sex chromosome. If the long condition is due entirely to a sex-linked gene we

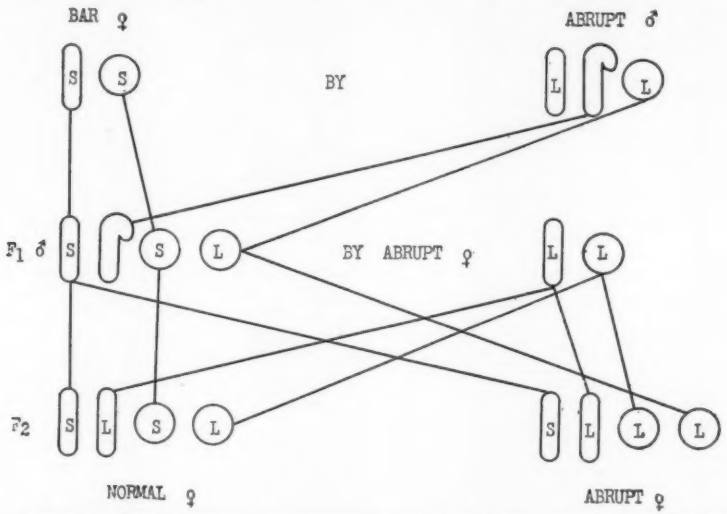

FIGURE 8.—Showing origin of sex chromosomes and third chromosomes in F_2 females from cross as indicated in figure 6.

would not expect any of these females to have a mean within the range of pure longs; for, as shown in figure 8, none of these females are homozygous for the X chromosome from the long stock. It has already been shown that the heterozygous condition has a range midway between those of the abrupt and bar stocks. Since some of these females (in fact half) show a mean within the range of the abrupt stock it seems probable that there is also an autosomal gene involved in making the egg long. The dash line shown in figure 9 represents the range of ten females that show the abrupt character. These females, as shown in figure 8, receive both their second chromosomes from the abrupt (long) stock. Since there is evidence that an autosomal gene is involved in making the egg long and since we know

that the females showing the abrupt character receive both their second chromosomes from the abrupt (long) stock, their behavior should indicate whether the autosomal gene is in the second chromosome. If the autosomal gene involved is in the second chromosome, all of the females showing the abrupt character should have means which fall within the range of the abrupt stock. We find that some of these females have means which fall outside the known range of the abrupt stock, showing that the

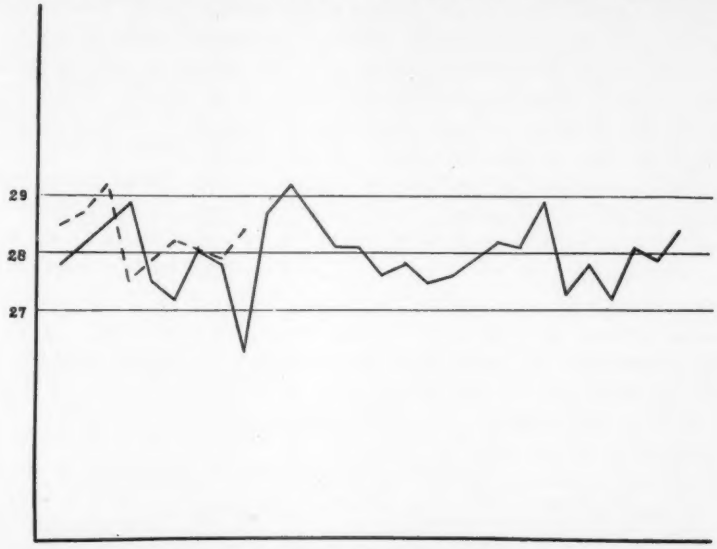

FIGURE 9.—Range of means of individual females from back-cross of F_1 male (from cross of bar female by abrupt male) to an abrupt female. The solid line indicates range of all F_2 females, and the dash line, the segregated abrupt females.

most important autosomal gene increasing the length of the egg is not in the second chromosome.

Thus from this back-cross we have three lines of evidence as to the genes involved in producing the long egg condition found in abrupt stock:

1. When the F_2 females carry only one X chromosome from the abrupt (long) stock, half of them show a mean which falls below the range of the abrupt stock. This substantiates the previous indication, that one of the main factors producing the long egg is in the sex chromosome.

2. Since about half of these females have means which fall within the range of abrupt stock, it seems probable that there is also an important autosomal gene involved which makes the egg long.

3. The most important autosomal gene involved in making the egg long is not carried by the second chromosome.

LINKAGE DATA FOR THE ABRUPT STOCK (LONG)

Since evidence was found that at least one gene involved in making the egg long, is carried by an autosome, the following linkage experiments were planned to locate more accurately this gene. In testing the linkage group to which such autosomal genes might belong, abrupt females were out-crossed to three stocks each of which was known to carry a factor for one of the three autosomal linkage groups. Purple was used in the second chromosome, cardinal in the third, and eyeless in the fourth. The abrupt stock was out-crossed to each of these stocks, and the F_1 male was back-crossed to these respective stocks. Purple has a mean of 26.2 units, cardinal a mean of 26.0, and eyeless a mean of 27.8. These means were computed from the measurement of two-hundred eggs. It will be observed that all of the mutations to which abrupt is back-crossed show an egg size less than that of abrupt. In the F_2 of such back-crosses, eggs were measured from females showing the character of the stock to which abrupt was out-crossed and also from females lacking this mutation. Thus a female known to be homogametic for the origin of a given chromosome pair is compared with a female known to be heterogametic for the origin of the same chromosome pair. The former receives both members of a given pair of chromosomes from the stock to which abrupt is out-crossed and the latter carries one member of this pair from the abrupt stock. Thus, if the back-cross is made to the chromosome group to which a "long" autosomal gene belongs, the females which show the mutation of this group should have a shorter egg length than the normal females; for, the normal females are known to possess one chromosome of this pair from the abrupt (long) stock. Figure 10 shows the origin of the pair of autosomes in which the long gene is located, for these two types of females.

In crossing any two stocks which differ in egg size the usual range of the F_1 females is mid-way between the two. However, there has been observed some variation in the range of F_1 flies. This fluctuation is probably due to the effect of some environmental factor not determined. This variation is a possible source of error in interpreting the results, so that slight differences have not been considered as significant. Table 6 gives these data. It will be seen that in the out-cross to the fourth linkage group (eyeless) the normal and eyeless from the F_2 generation show ranges that are practically identical. The cross to the second linkage group (purple) shows some difference in range between the two types of females. In the cross to the

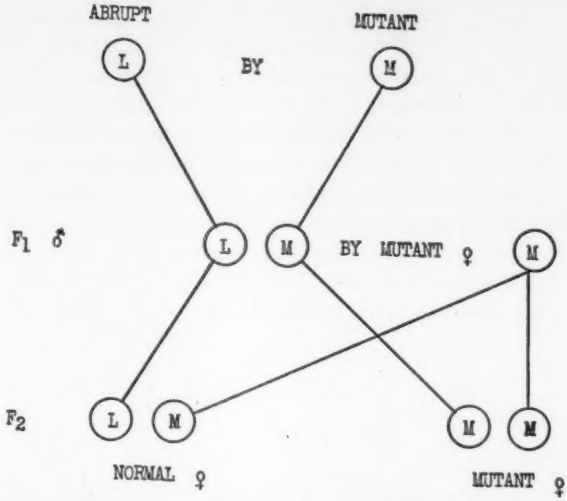

FIGURE 10.—Showing the origin of any pair of autosomes which may differ as to genes influencing egg length in the two stocks crossed. This type of cross was made, utilizing recessive mutants in each linkage group, in determining the linkage relations of the abrupt stock.

TABLE 6

Linkage relations of long egg condition in abrupt stock.

Cross to second group

Units	24	25	26	27	28	29	30	31	Mean	χ^2	P
Purple female		19	65	77	35	4			26.7	31.04	.000005
Normal female		6	42	65	79	8			27.2		

Cross to third group

Cardinal female	9	95	81	14	1				25.5	294.76	.000000
Normal female		2	14	62	95	27			27.7		

Cross to fourth group

Eyeless female				14	60	90	36		28.7	3.76	.3
Normal female			1	14	77	80	28		28.6		

The application of the χ^2 test indicates that the difference found in the tests with the second and third linkage groups is significant. Thus, there are genes in the second and third chromosomes which affect egg size.

third linkage group there is a rather striking difference in range between the normal and cardinal females. There is here no evidence that there are any genes in the fourth chromosome which influence egg size. There is some evidence that the second chromosome bears one or more genes which increase the length of the egg and very strong evidence that the most important gene or genes for increasing the length of the egg are in the third chromosome. The application of the χ^2 test indicates that the differences found in the case of the second and third linkage groups are significant. The principal autosomal factor for which we had previously found evidence (figure 9) is probably in the third linkage group and there is also a minor modifier which belongs to the second linkage group.

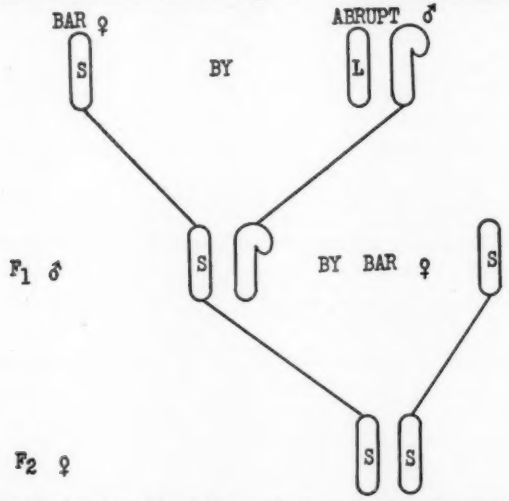

FIGURE 11.—Showing the origin of the sex chromosomes in the females from the cross as indicated in figure 9.

From the foregoing experiments with the abrupt stock we have evidence that there are two principal and one minor factor involved in making the egg long. One of the major factors is sex-linked and the other belongs to the third linkage group. A minor factor showing linkage to the second group slightly affects the length of the egg. The effects found may be due to a single gene or to the combined action of several genes which have accumulated in particular chromosomes.

GENETIC BEHAVIOR OF THE SHORT EGG OF BAR STOCK

A bar female was crossed to an abrupt male and the resulting F_1 male

was back-crossed to a bar female. This cross was made to test whether any genes involved in producing the short egg which is characteristic of bar, is sex-linked. Figure 11 shows the origin of the sex chromosomes in these F_2 females. In this cross, the counteracting influence of the "long-producing" factors carried by the abrupt stock, must be considered. However, since here both of the sex chromosomes of the F_2 female come from the bar stock, we have eliminated the sex-linked "long" gene of abrupt. Half of these females will carry in one of their third chromosomes a "long-producing" gene from the abrupt stock. The other half of these

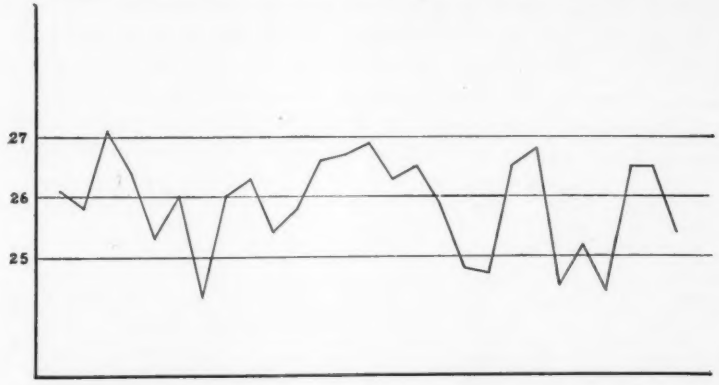

FIGURE 12.—The range of means of individual females from the back-cross of an F_1 male from the cross bar female by abrupt male, to a bar female. Since many of these means fall outside the range of pure bars, the most important genes for the short egg are not in the sex chromosome.

F_2 females should be free from the effects of either of the two "long-producing" genes and since both of their sex chromosomes come from the bar (short) stock, they should be homozygous for any "short-producing" sex-linked genes. By comparison of figure 12 with figure 3 it will be seen that considerably less than half of these females have a mean which falls within the range of pure bar. It seems probable therefore that there are no sex-linked factors which produce the short egg condition of bar.

If there were a single autosomal gene which makes the bar egg short, then half of these F_2 females should be homozygous for this gene and should have the range of pure bars. However, the counteracting effect of the "long" autosomal gene must be kept in view. Half of these females also carry this gene and with the free assortment of these two genes we would expect one-fourth of these F_2 females to be homozygous for the

"short" gene and free from the effects of the "long" gene. This in fact, is about the proportion of pure "shorts" that are realized.

It seems therefore that there is a single major autosomal factor that produces the short condition of bar.

LINKAGE DATA FOR THE BAR STOCK (SHORT)

The linkage behavior of the short condition was tested in the same way as in the case of abrupt. Here it was possible to use dominant mutations in each group. The F_1 males showing the dominant mutation were back-crossed to the bar stock, and in the F_2 generation, females showing the dominant mutation were compared (as to egg size) with females lacking this character. The chromosomal complexes of both types of females are shown in figure 13. Here the F_2 females which show the dominant mutation will receive from the bar stock but one member of the pair of chromosomes under consideration, while in the case of the females lacking the

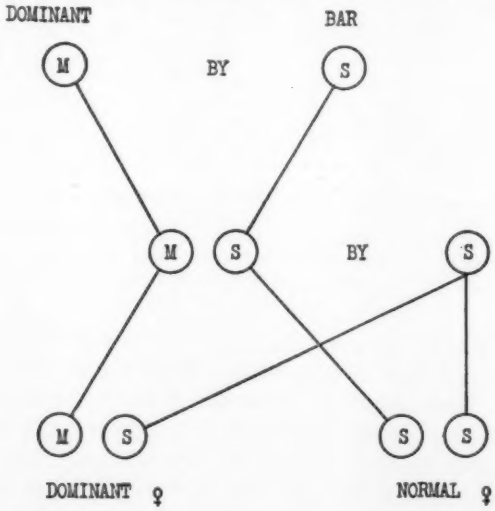

FIGURE 13.—Showing the origin of autosomes in crosses made to determine the linkage relations of the bar stock.

dominant character, both chromosomes of this pair must come from the bar stock. Since all of the stocks used averaged considerably larger than the bar stock, the females showing the dominant character in the group to which the "short" autosomal factor belongs, should average larger than

the normal females. In crosses to linkage groups in which the "short" autosomal factor does not belong, there should be no difference between the two types of females. Curly and lobe$_2$ were used for the second chromosome and dichaete for the third. Since both the characters, bar and eyeless, affected the shape of the eye, it was not possible to obtain a test for the fourth linkage group. These linkage data are shown in table 7. There is

TABLE 7

Linkage data for short egg condition in bar stock.

Cross to second group

Units	22	23	24	25	26	27	28	29	Mean	x^2	P
Lobe$_2$ female		1	11	85	85	18			25.5	114.84	.000000
Normal female		9	53	126	12				24.7		
Curly female			4	37	76	73	9	1	26.5	108.52	.000000
Normal female			14	87	75	21	3		25.6		

Cross to third group

Dichaete female			10	80	83	27			25.6	20.48	.001250
Normal female		2	29	94	57	17	1		25.3		

The differences found in the tests with the second linkage group indicate that there are genes in the second chromosome which influence egg length. It is questionable whether the difference found in the case of the third group is significant.

evidence for one or more genes in the second chromosome which decrease the length of the egg. This is shown by the two crosses, to curly and to lobe$_2$, for, in both cases the females known to be heterogametic for the origin of their second chromosomes average considerably larger than those females known to be homogametic for the same pair of chromosomes, both members of which have originated in the bar stock. There is a similar difference in the cross to dichaete, a third-chromosome dominant, but here the difference is rather slight and, if significant at all, is indicative of a minor gene affecting egg size. The evidence for a "short" gene in the second chromosome is substantiated by data given earlier in figure 7. It was shown that segregated abrupt (second-chromosome) females produce eggs that average longer than those lacking this character and one possible explanation of this would be that the second chromosome of the bar stock carried one or more genes which decreased the length of the egg.

Thus, it appears that we have in the second chromosome one or more genes which largely determine the shortness of the bar egg and there may be in addition a minor modifier in the third chromosome.

THE GENETIC BEHAVIOR OF APRICOT-VERMILION-FORKED STOCK

The stock apricot-vermilion-forked has an egg mean of 28.5 units. This is seen to be almost as extreme as abrupt. If the two stocks are crossed the F_1 females show a range more or less intermediate between the two. These data are shown in table 8. Since the F_1 mean is so near that of the two parent stocks, it is evident that the two stocks must have in common some of the principal factors which determine the long egg condition.

TABLE 8

Results of crossing two long-egg stocks, abrupt and w_a v f.

STOCKS	NUMBER OF EGGS MEASURED	UNITS AND FREQUENCIES						MEAN
		26	27	28	29	30	31	
w_a v f	500	3	54	190	181	68	4	28.5
F_1 of w_a v f by abrupt	200		11	84	73	31	1	28.6
Abrupt	500		8	147	214	117	14	29.0

Since the range of egg length in the F_1 generation of the cross abrupt by w_a v f is so near that of the two stocks crossed, it is assumed that some of the principal genes influencing egg length in the two stocks are identical.

In the cross of apricot-vermilion-forked to dichaete (table 9) the F_1 male was back-crossed to an w_a v f (apricot-vermilion-forked) female. Dichaete has an egg range much shorter than w_a v f but the F_2 females, receiving both X chromosomes from the w_a v f stock, showed a range practically identical with that of the pure w_a v f stock. This would indicate that here,

TABLE 9

Comparing range of w_a v f stock with F_2 females from back-cross of F_1 male in cross w_a v f female by dichaete male, to w_a v f female.

STOCKS	NUMBER OF EGGS MEASURED	UNITS AND FREQUENCIES						MEAN
		26	27	28	29	30	31	
w_a v f	500	3	54	190	181	68	4	28.5
F_2 females from back-cross	400	8	73	184	112	23		28.2

The similarity in range of the two types of females tested indicates that some of the principal genes determining egg length are sex-linked.

as in abrupt, a sex-linked gene plays an important part in making the egg long.

Having evidence that both w_a *v f* and abrupt carry in the sex chromosome an important gene for their long egg condition, linkage experiments were carried out with w_a *v f* to determine its autosomal condition with respect to egg size. Table 10 gives the results of these linkage crosses. w_a *v f* was crossed to purple, cardinal and eyeless, recessive mutants from the second, third and fourth chromosome groups, respectively. These crosses were made in the same manner as were the crosses in the case of abrupt. There was found no evidence for any genes in the second chromosome which modify the length of the egg. In the test with the fourth linkage group the difference between the two types of females is significant

TABLE 10

Linkage data for long egg condition in w_a v f stock.

Units	23	24	25	26	27	28	29	30	Mean	x^2	P
Cross to second group											
Purple female	2	11	92	74	20	1			25.5	6.92	.135888
Normal female	1	6	73	94	26				25.7		
Cross to third group											
Cardinal female			51	64	58	25	2		26.3	16.54	.002
Normal female		1	22	78	62	30	7		26.6		
Cross to fourth group											
Eyeless female			8	40	73	59	18	2	27.2	57.12	.000000
Normal female			3	9	42	83	53	10	28.0		

There is a significant difference between the two types of females in the test with the fourth linkage group, and in this group only is there unquestionable evidence for factors influencing egg length.

and indicates that there are one or more genes in the fourth chromosome which modify the length of the egg. The evidence for any modifiers carried by the third chromosome is questionable. Thus it seems that in the w_a *v f* stock we have the same sex-linked modifiers that exist in the abrupt stock, but the autosomal genes involved are not the same as those found in the abrupt stock. It seems that the second-chromosome genes found effective in the case of abrupt are here lacking. This is quite

possible for it will be shown that the sex-linked gene or genes in abrupt have alone the same effect that they have in conjunction with the autosomal genes.

SHORT EGG SIZE OF RUBY STOCK

The mean for ruby, computed from the measurement of five-hundred eggs, was found to be 25.4 units. It shows a range much shorter than the average but the mean is somewhat higher than that of bar (mean 24.7).

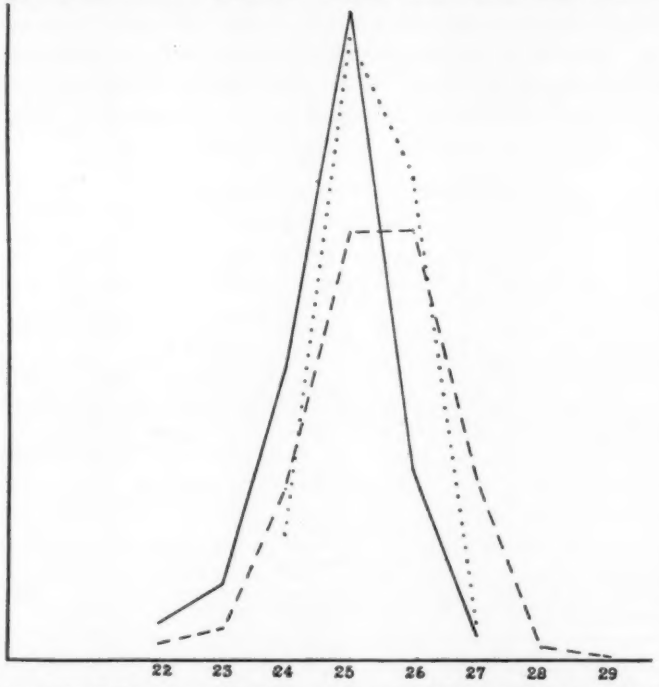

FIGURE 14.—Results of crossing the two short-egg stocks, bar and ruby. The solid line indicates the range for bar; the dash line, for ruby; and the dotted line, for the F_1 generation from crossing the two.

To determine whether the same factors were involved in the two "short" stocks, they were crossed (see figure 14). It is seen that the F_1-generation size range is also short as in the two parental stocks. It follows that the principal factors which make bar short must also be carried by the ruby stock. However, since the ruby stock consistently ranges slightly higher than bar, it must have a somewhat different genetic constitution. The

ruby stock was selected for ten generations, breeding only from the shortest eggs and the results are shown in table 11. It is seen that selection reduced the range of the ruby stock to practically that of bar. Thus by selection it has been possible to eliminate the factor or factors which tended to slightly increase the egg size of ruby.

TABLE 11

Effect of selection in ruby stock.

STOCKS	NUMBER OF EGGS MEASURED	UNITS AND FREQUENCIES								MEAN
		22	23	24	25	26	27	28	29	
Ruby stock.....	500	7	13	68	167	168	71	5	1	25.4
Ruby (selected).	200		1	23	134	42				25.1
Bar stock.....	500	14	30	113	257	77	9			24.7

It is seen that selection was effective in reducing the length of the egg of the ruby stock.

F_1 BEHAVIOR IN CROSSES OF VARIOUS STOCKS

In the crosses between various stocks, the range of egg size of the F_1 flies is of interest in that there is a general tendency for the mean of egg size to be intermediate between the two parental stocks. The nearness

TABLE 12

The F_1 behavior in crosses of various stocks.

STOCKS	BAR MEAN, 24.7		w_a *v f* MEAN, 28.5		ABRUPT MEAN, 29.0	
	Stock mean	F_1 mean	Stock mean	F_1 mean	Stock mean	F_1 mean
Bar.....					24.7	27.1
Ruby.....	25.4	25.3				
Lobe$_2$.....	26.5	25.5	26.5	27.4	26.5	27.2
Dichaete....	26.8	25.9	26.8	28.1	26.8	27.4
w_a *v f*.....					28.5	28.6
Abrupt.....	29.0	27.1	29.0	28.6		

This table shows that the F_1-generation range is always intermediate between any two stocks crossed.

with which the F_1 mean approaches the median between the two stocks is rather striking. Table 12 gives the results of these crosses. A series of crosses are shown for bar, w_a *v f* and abrupt. The column to the left shows the different stocks to which they were crossed. These stocks are arranged in order of their mean sizes beginning with the short-egg

stock, bar, and ending with abrupt (long). In adjacent columns are given the means of the stock to which these three stocks were crossed and the mean of the F_1 generation of each cross. Examination of the table will show that the mean of the F_1 generation is always intermediate between any two stocks crossed. It will also be seen that when any stock is crossed to a series of stocks which successively increases in size, the F_1 means increase in the same manner, although not exactly in the same ratio. This regular intermediate nature of the F_1 generation is of interest. Such a behavior would be expected if we had here a number of rather small modifiers for egg size. The stocks varying in range would each have a different combination of them, and the F_1 generation from crossing such stocks would be intermediate. From the foregoing experiments we have found some evidence for small modifying factors.

GENERAL DISCUSSION

Measurements of various stocks of flies showed that each seemed to have an egg size of its own. No one condition was found which might be called "wild-type." The sizes found to be characteristic of the different races were very constant and seemed to be in no way affected by any environmental conditions tested. They were also not correlated with any size or age differences in the female.

Conditions similar to those found here with respect to independence of egg size and size of female, have been described in other forms. The smaller breeds of the domestic fowl frequently produce larger eggs than do some of the larger breeds. Here also the size difference of the egg is due to genetic constitution rather than environmental conditions. EAST (1913) found in Nicotiana that the corolla spread of the flower practically showed independence of the size of the plant.

If the nature of the size character could be traced to its last analysis, it would probably be found that we are dealing with some anatomical relation of the female reproductive organs which govern the formation of the egg. If so, in the study of egg size we are dealing with a condition which is the result of this character. However, the character and its result are both expressions of the same genetic constitution, and the results are in no way altered.

In two stocks selection was carried out for ten generations. These stocks were abrupt and ruby. In the case of the former the strain was carried by selecting only the very longest eggs, and in the latter only the shortest eggs were used. The selection in the abrupt stock showed no increase in egg size. This is more or less to be expected since the stock was,

to begin with, the most extreme stock examined. In the case of ruby it was possible by selection to reduce the egg size to a range practically identical with that of bar. Table 11 gives these results. It was possible to eliminate whatever genetic differences existed between bar and ruby.

It was possible to locate in all four chromosomes, genes that affect egg size. The factors found were quite variable in their effect upon egg length. It is not known whether the effects correlated with particular chromosomes are due to a single gene or to the action of several genes in that chromosome. From the F_1 behavior (discussed in the last section) of various stocks it seems probable that there may be many minor factors, each having only a very slight effect, which contribute to the variations found in egg size. The results of selection of the ruby stock indicate that there must have been one or more minor factors which it was possible to eliminate. By linkage experiments it was possible to locate some of these minor factors.

In discussing the foregoing linkage experiments it has been stated that the stocks, abrupt, bar and $w_a\, v\, f$, each carries specific genes which increase or decrease the length of the egg. This is perhaps an overstatement of what has actually been determined, for in this study no wild type is known and very little is really known as to the factors for egg size carried by the stocks to which the above-mentioned races were crossed in determining the linkage relations. The linkage experiments indicate only that there are, with respect to egg size, certain factor differences between the stocks crossed, and just how the factors in any linkage group react upon the length of the egg, is unknown. The fact of chief interest, however, is that there exist these genes which are responsible for the variations in the length of the egg.

In determining the long egg length found in the abrupt stock two major factors were found, one sex-linked and the other belonging to the second group of linked characters. In the F_2 generation of the cross, bar female by abrupt male (figure 4), we obtained an individual with an egg mean typical of pure abrupt. This shows that the autosomal gene in abrupt is capable of giving the extreme abrupt condition without the aid of the sex-linked gene, for here both sex chromosomes came from the bar stock. This segregated "long" must have received both of its second chromosomes from the abrupt stock, for the F_1 behavior of this cross shows that a fly heterozygous for both the sex-linked and autosomal factors never shows the extreme range of pure abrupt. In figure 6 we have evidence that the sex-linked factor in abrupt stock is capable, in the presence of the autosomal factor in a heterozygous condition, of producing the pure abrupt

size. Here all the females are homozygous for the sex-linked factor, but about half of them must be heterozygous for the autosomal factor. However, all of them have the range of pure longs.

Due to the presence of the two major factors in abrupt, each of which in the absence of the other is probably capable of producing the long egg condition, the effects of the "short" gene in bar was frequently submerged in the crosses shown. However, in figure 6 we see that females heterozygous for the "short" factor from bar, show a lower range than those known not to carry this gene in either chromosome. Thus the "short" factor in a heterozygous condition slightly counteracts the effect of the sex-linked factor in a homozygous condition.

In a back-cross of an F_1 male from the cross, abrupt female by bar male, to a bar female, a few F_2 females were examined for egg size (data not given). Only six were measured, but two of these showed the range of pure shorts. Here all females received from the abrupt stock one sex chromosome which carried the "long" sex-linked gene. It follows that the "short" factor of bar in a homozygous condition produces its full effect even in the presence of the counteracting influence of the "long" factor in a heterozygous condition.

We have dealt with variations that are exceedingly minute. The most extreme variants considered have shown a difference of less than .2 millimeters. Nevertheless it has been possible to show that a number of genes are involved in bringing about these variations. To what an extreme degree of minuteness a genic change may be manifested is one of the interesting results of this study.

In conclusion, I wish to express my gratitude to Prof. T. H. MORGAN for valuable advice and criticism during the course of this study. I am also indebted to Dr. A. H. STURTEVANT for many helpful suggestions.

SUMMARY

1. Of the various stocks examined, many show characteristic and constant ranges, for egg size. No one condition found could be classed as "wild-type."

2. The factors for egg size proved to be entirely independent of those responsible for mutations in the races where the unusual sizes were found.

3. Egg size is, as far as tested, independent of external conditions. It is also independent of the size and age of the female which lays the egg.

4. Factors for egg size were found in all four linkage groups.

5. From the study of the abrupt stock, genes which influence the size of the egg, were located in the first, second and third chromosomes.

6. Linkage experiments with the bar stock indicate that there are one or more genes in the second chromosome which affect the size of the egg.

7. Genes affecting egg size were located in the first and fourth chromosomes from tests with the $w_a\ v\ f$ stock.

8. In crossing any two stocks, the egg size of the F_1 females was almost invariably intermediate between those of the two parent stocks.

9. There is evidence that in addition to the factors already mentioned, there are several minor ones which have a slight influence upon the size of the egg.

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AN EFFECT OF X RAYS ON THE LINKAGE OF MENDELIAN CHARACTERS IN THE SECOND CHROMOSOME OF *DROSOPHILA MELANOGASTER*

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INTRODUCTION

In previous contributions it has been shown (MAVOR 1923 a, b) that X rays cause a decrease in crossing over between eosin eye color and miniature wing in the first or sex chromosome of *Drosophila melanogaster*. Since PLOUGH (1917) has shown that temperatures above or below normal cause an increase in crossing over in the black-to-curved region of the second chromosome in the same species it appears of interest to discover what, if any, effect X rays have on crossing over in the same region of the second chromosome. The investigation to be reported here had this in view.

When a normal wild-type fruit-fly (*Drosophila melanogaster*) is mated with a black-bodied, purple-eyed and curved-winged fly, the heterozygous offspring obtain the factors for the three mutant characters from one parent. If, now, the daughters of such a cross are back-crossed to black purple curved males, a majority of their offspring will be either wild-type or black purple curved, showing that these characters are linked (in this

case in the second chromosome). These two kinds of flies form the non-crossover classes. There will be also a number of offspring in which the characters are not associated in this way, namely, normal-bodied, purple-eyed and curved-winged, and black-bodied normal-eyed and normal-winged (crossing over between black and purple); normal-bodied, normal-eyed and curved-winged, and black-bodied, purple-eyed and normal-winged (crossing over between purple and curved); and normal-bodied, purple-eyed and normal-winged, and black-bodied, normal-eyed and curved-winged (double crossing over). The percentages of crossing over between the characters mentioned have been accurately determined independently by BRIDGES, MULLER and PLOUGH, and a summary of their results is given by BRIDGES and MORGAN (1919). They give as the weighted averages of all previous determinations for crossing over between black and purple, 6.2 percent, the determination having involved somewhat over 50,000 flies, and for crossing over between purple and curved, 19.9 percent, this determination having involved somewhat over 60,000 flies. It should be added that the determinations by the different investigators are in approximate agreement.

DESCRIPTION OF EXPERIMENTS

The investigation to be described in the present paper included two experiments. In the first of these, twenty sisters from the mating of a wild-type female with a black purple curved male were used. Eleven were kept as controls and nine were X-rayed for 3 minutes and 15 seconds at a distance of 23.5 cm from the tungsten target, the Coolidge tube being operated at 50,000 volts and .05 amperes. Previous experiments had shown that the temperature in the X-ray box in which the flies were exposed did not vary from that of the room by more than 1°C. On the day after the X-raying, all of the twenty females were mated to black purple curved males and placed in individual culture bottles. The pairs, control and X-rayed, were changed to new bottles every three days until the eighteenth day when they were killed. The offspring coming out in the bottles were counted daily until the seventeenth day after mating. The second experiment was performed in the same way with the following exceptions: the control contained eleven pairs and there were twenty-seven pairs in which the females were X-rayed. The X-ray treatment was the same, except that the time was shortened to 3 minutes. The females were mated immediately after being X-rayed and were transferred to new bottles every three days until the twelfth day, when they were killed. The flies coming out in the culture bottles were counted every three or

four days until the seventeenth day after mating. The bottles of both experiments were kept in an incubator set at 22°C, and during the whole time the temperature did not go below 20°C, or above 24°C.

First experiment

The results of the first of these experiments, our number 103, are shown in table 1. The data from which the percentages in this table were calculated are given in the appendix in table 5. The sterilizing effect of the X

TABLE 1

Experiment 103. Effect of X rays on crossing over in the second chromosome. The numbers in all of the columns except those headed $\frac{Diff.}{P.E._{diff.}}$ *give percent of crossing over.*

Black–to–purple region

BOTTLE	X-RAYED	CONTROL	DIFFERENCE	P. E. $_{diff.}$	$\frac{DIFF.}{P.E._{diff.}}$
First		5.6			
Second	18.5	6.3	12.2	2.1	5.9
Thi d	24.4	4.4	20.0	1.0	19.1
Fourth	17.3	3.5	13.8	1.0	13.8
Fifth	9.5	3.3	6.2	1.1	5.8
Sixth	8.6	4.2	4.4	1.5	3.1

Purple–to–curved region

BOTTLE	X-RAYED	CONTROL	DIFFERENCE	P. E. $_{diff.}$	$\frac{DIFF.}{P.E._{diff.}}$
First		17.2			
Second	26.2	14.7	11.5	3.0	3.8
Third	28.4	14.7	13.7	1.8	7.5
Fourth	16.2	16.2	0.0	2.0	0.0
Fifth	20.4	15.8	4.7	2.2	2.1
Sixth	14.0	16.1	−2.1	2.6	0.8

rays was so marked in the first bottles, only 12 offspring being produced, that an accurate crossover value cannot be obtained. In the second bottles there is a conspicuous difference in the case of the first crossover (that between black and purple). This difference 12.2, divided by the probable error, 2.07, is equal to 5.9. The difference divided by the probable error for the purple-to-curved crossover is 3.8. In the third bottles the greatest difference occurs. Here the crossover value has been increased from that of the control, 4.39 percent, to 24.36 percent, giving a difference of

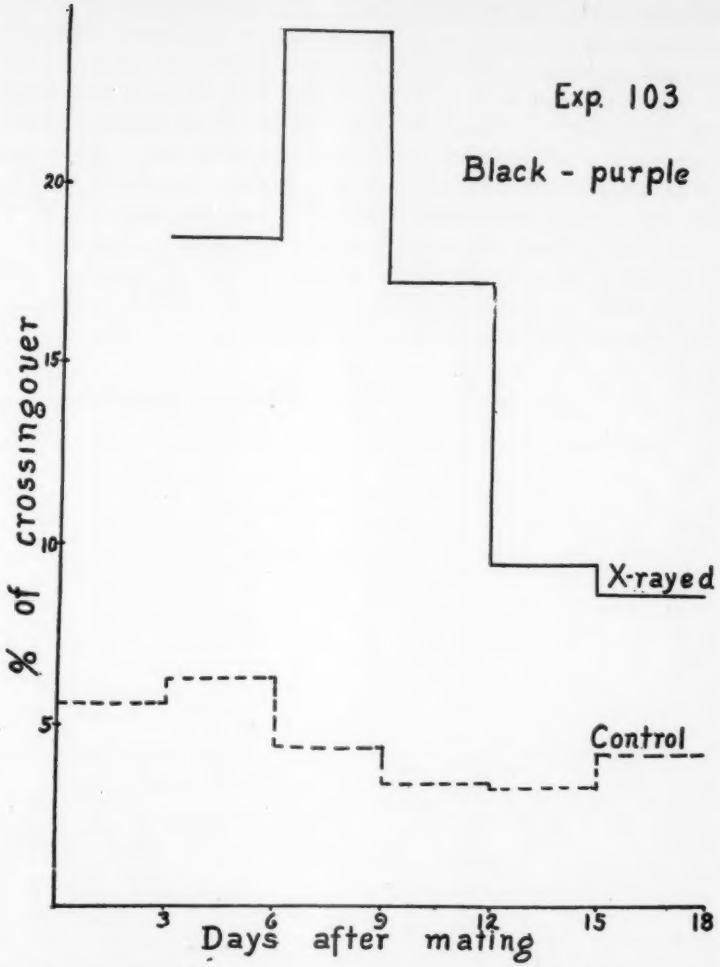

FIGURE 1.—Graph of percent of crossing over between black (body color) and purple (eye color) in experiment 103. The X-rayed females were treated soon after emergence from the pupa (within two days). Both control and X-rayed females were mated on the day after the X-ray treatment was given. The pairs of flies, both in the X-rayed and control sets were transferred to new bottles every three days. In making the graph the percentages were determined from the total number of F_1 which emerged in the bottles within seventeen days from the time the parents were placed in the bottles. The number of F_1 which emerged in the first bottles of the X-rayed females was not sufficient to give a significant crossover value.

approximately 20 percent; this divided by the probable error gives 19.1. For the purple-to-curved region in the third bottles the difference between the X-rayed and control, divided by the probable error, is 7.5.

The statistical treatment of the data shows that there is a significant increase in crossing over in the X-rayed females for the black-to-purple crossover in each of the second, third, fourth and fifth bottles. In the case of the purple-to-curved crossover there is a significant increase in the second and third bottles and possibly the fourth. Since the purple-to-curved

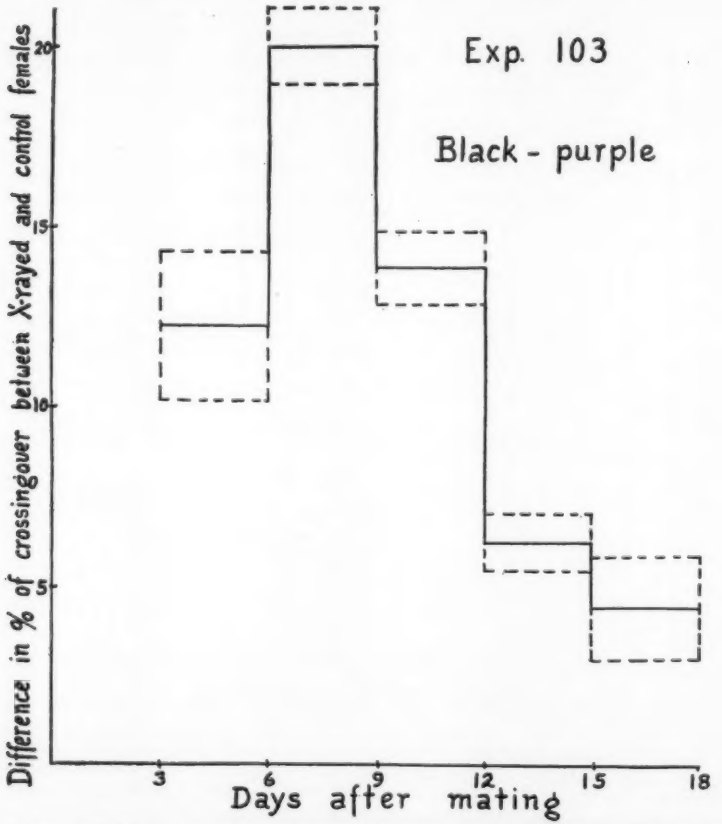

FIGURE 2.—Graph of crossing over between black and purple in experiment 103 using the same data as in figure 1, but plotted to show the difference (continuous line) in percent of crossing over between the X-rayed and control females and the probable error of the difference (interrupted line).

region has normally the larger crossover value it is clear that X rays have more effect on crossing over in the black-to-purple than in the purple-to-curved region. The percent of crossing over of black-to-purple in this experiment is shown graphically in figure 1. In figure 2 the difference in percent of crossing over between the X-rayed and control females is shown, the probable error being indicated by the broken lines.

Second experiment

The results of the second of these experiments, our experiment number 404, are shown in table 2. The data from which the percentages in this table were calculated is given in the appendix in table 6. The X-ray dose in this experiment was somewhat less than in the previous one, having

TABLE 2

Experiment 404. Effect of X rays on crossing over in the second chromosome. The numbers in all of the columns except those headed $\frac{Diff.}{P.\ E._{diff.}}$ *give percent of crossing over.*

Black–to–purple region

BOTTLE	X-RAYED	CONTROL	DIFFERENCE	P. $E._{diff.}$	$\frac{DIFF.}{P.\ E._{diff.}}$
First	4.8	4.2	0.6	0.8	0.8
Second	7.0	2.9	4.1	0.7	6.6
Third	14.7	3.6	11.1	0.5	24.4
Fourth	10.8	4.2	6.6	0.5	14.3

Purple–to–curved region

BOTTLE	X-RAYED	CONTROL	DIFFERENCE	P. $E._{diff.}$	$\frac{DIFF.}{P.\ E._{diff.}}$
First	19.8	18.6	1.2	1.5	0.8
Second	17.6	12.3	5.3	1.0	4.8
Third	23.6	12.9	10.7	0.8	12.5
Fourth	17.8	14.7	3.1	0.8	3.8

lasted only 3 minutes as against 3 minutes and 15 seconds in the case of the first experiment. Also a greater number of females were X-rayed. For this reason the probable errors of the differences are considerably smaller. The results of this experiment confirm those of the first. The differences are smaller, however, corresponding to the smaller dose of X rays given to the females. Considering first the black-to-purple region, one sees that, as in experiment number 103, in the first bottles there is no sig-

nificant difference between the percent of crossing over in the X-rayed and control females, $\frac{\text{Diff.}}{\text{P.E.}_{\text{diff.}}} = 0.8$. In the second, third and fourth bottles there is a significant difference, the greatest difference being in the third bottles, where the difference, 11.1, is 24.4 times the probable error of the difference. In the case of the purple-to-curved region in the first bottles

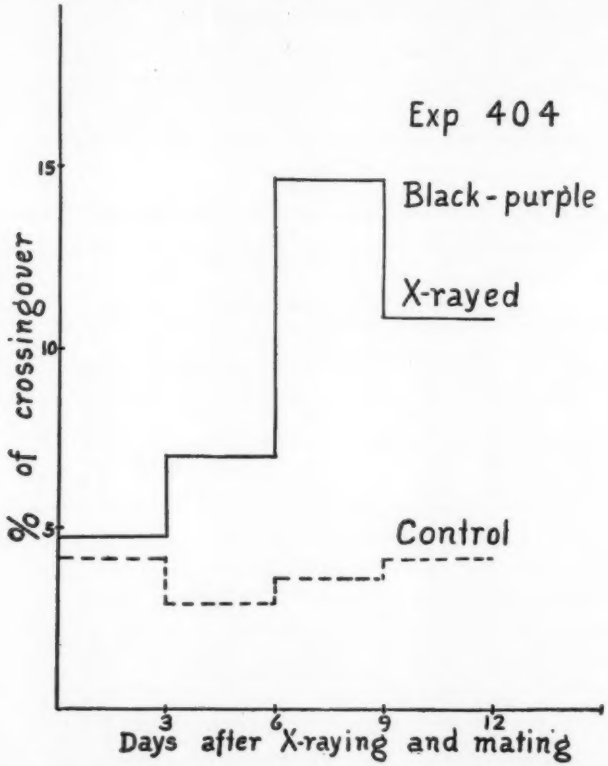

FIGURE 3.—Graph of crossing over between black and purple in experiment 404. The conditions of this experiment were similar to those of experiment 103, figure 1, with the exception that the females were mated immediately after X-ray treatment, and the X-ray dose was less.

there is also no significant difference between the percent of crossing over in the X-rayed and control females. In the second and third bottles the difference for the purple-to-curved region is significant, being greatest in the third bottles, where the difference is 12.5 times the probable error.

The difference in the fourth bottles is not significant. Graphs for this experiment for the black-to-purple region, similar to those for the first experiment, are shown in figures 3 and 4. Similar graphs for crossing over in the purple-curved region for the second experiment, No. 404, are shown in figures 5 and 6.

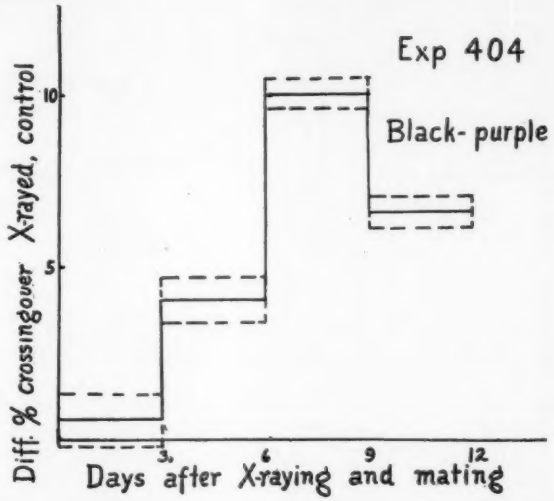

FIGURE 4.—Graph of crossing over between black and purple in experiment 404, plotted according to the method used in figure 2.

The combined results of the two experiments

The results of the two experiments are combined in table 3. Here it is seen that when the experiments are considered together there is a significant difference between the crossover values of the control and X-rayed females for the black-to-purple region in the second, third and fourth bottles, and for the purple-to-curved region in the second and third bottles and possibly the fourth. A graph of the weighted values of the percent of crossing over between black and purple in experiments 103 and 404 for three-day intervals (each set of bottles) is shown in figure 7. In figure 8 is shown a similar graph for the difference in percent of crossing over between the control and X-rayed females, the interrupted lines indicating the corresponding probable errors. In figure 7 it is seen that the control, dotted line, varied from 4.78 percent to 3.25 percent, there being a slight decrease with age until the fifteenth day. This decrease in the crossover value with age has already been reported by BRIDGES (1915) and PLOUGH

(1917). The effect of the X rays on the crossover value (continuous line) becomes apparent in the counts of the offspring of the X-rayed females in the second bottles (the fourth to the seventh day after X-raying in the case of the first experiment and the third to the sixth day in the case of the

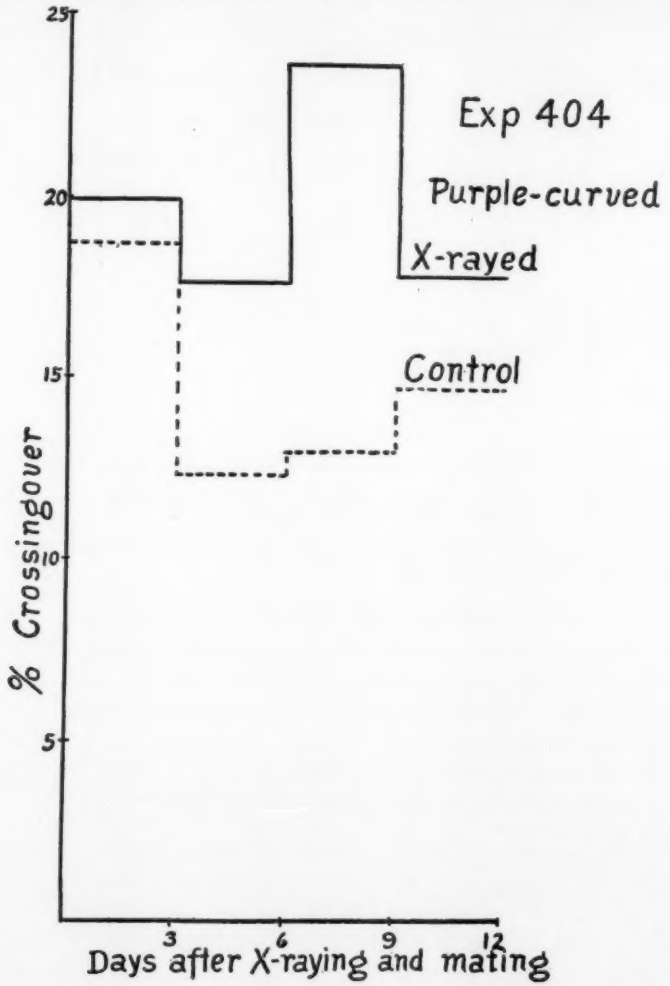

FIGURE 5.—Graph of crossing over between purple and curved in experiment 404, plotted as in figure 3.

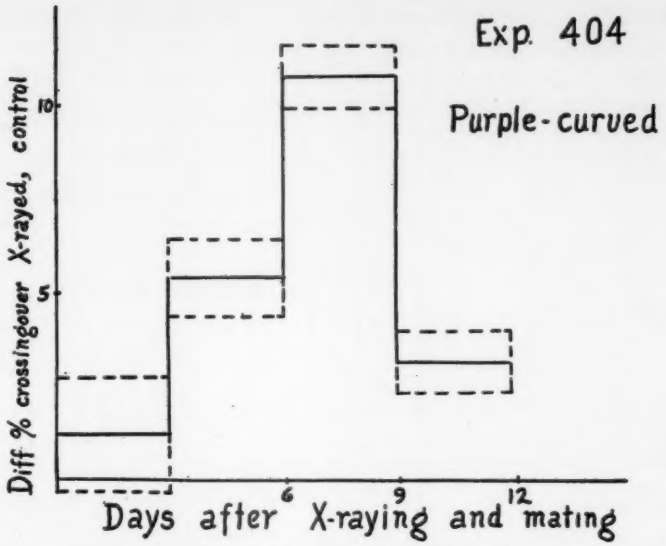

FIGURE 6.—Graph of crossing over between purple and curved, plotted as in figure 4.

TABLE 3

Experiments 103 and 404 combined. Effect of X rays on crossing over in the second chromosome. The numbers in all of the columns except those headed $\frac{Diff.}{P.\ E._{diff.}}$ *give percent of crossing over.*

Black–to–purple region

BOTTLE	X-RAYED	CONTROL	DIFFERENCE	P. $E_{diff.}$	$\frac{DIFF.}{P.\ E_{diff.}}$
First	4.7	4.8	−0.1	0.8	0.16
Second	8.0	4.5	3.5	0.6	6.11
Third	15.8	4.0	11.8	0.4	29.14
Fourth	11.2	3.8	7.4	0.4	20.78

Purple–to–curved region

BOTTLE	X-RAYED	CONTROL	DIFFERENCE	P. $E_{diff.}$	$\frac{DIFF.}{P.\ E_{diff.}}$
First	19.6	18.0	1.6	1.4	1.1
Second	18.4	13.5	4.9	1.0	5.2
Third	24.1	13.8	10.4	0.7	14.6
Fourth	17.7	15.5	2.2	0.7	3.3

second experiment). The effect of the X rays is greatest in the third bottles (seventh to tenth day after X-raying in the first experiment and sixth to ninth day after X-raying in the second experiment). The fourth, fifth and sixth bottles (ninth to eighteenth day) show a gradual recovery toward

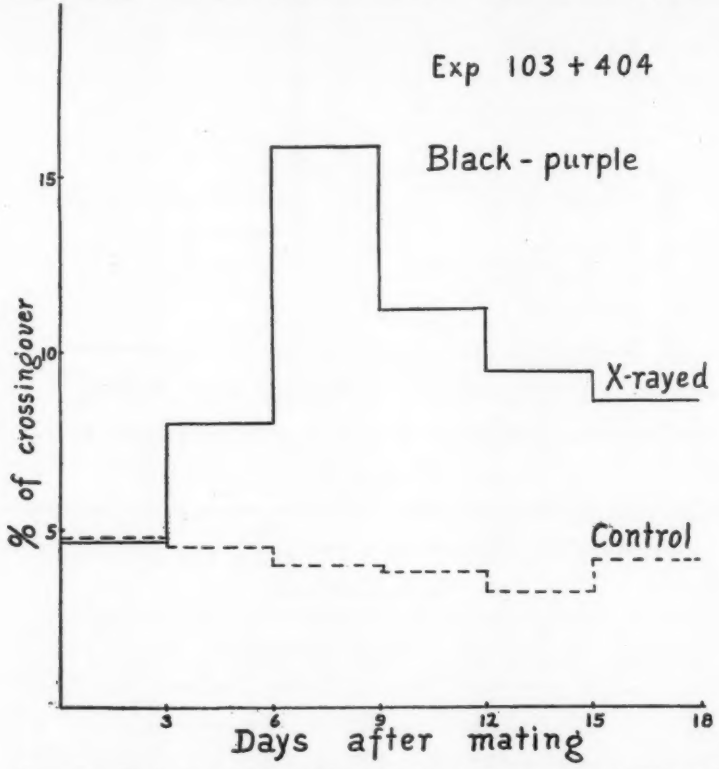

FIGURE 7.—Graph of crossing over between black and purple, experiments 103 and 404 being combined. It should be noted that the graph starts with the time of mating in each of the experiments and that the X-ray treatment was given in one case (experiment 103) on the day before mating and in the other case (experimeht 404) on the day of mating.

the crossover value of the control. The difference between the crossover value in the cultures of the X-rayed and control females divided by the probable error of the difference is for each of the six bottles 0.16, 6.11, 29.14, 20.78, 5.78 and 3.07, respectively.

The data obtained show that X rays have a similar effect on crossing over between purple and curved, although the increase in the crossover

value is relatively not so great as in the case of black to purple and recovery to the normal crossover value is more rapid. A graph showing the difference in percent of crossing over between purple and curved for experiments 103 and 404 (the values having been weighted) is shown in figure 9.

The values of the coincidence of crossing over between black to purple and purple to curved for the cultures of the X-rayed and control females

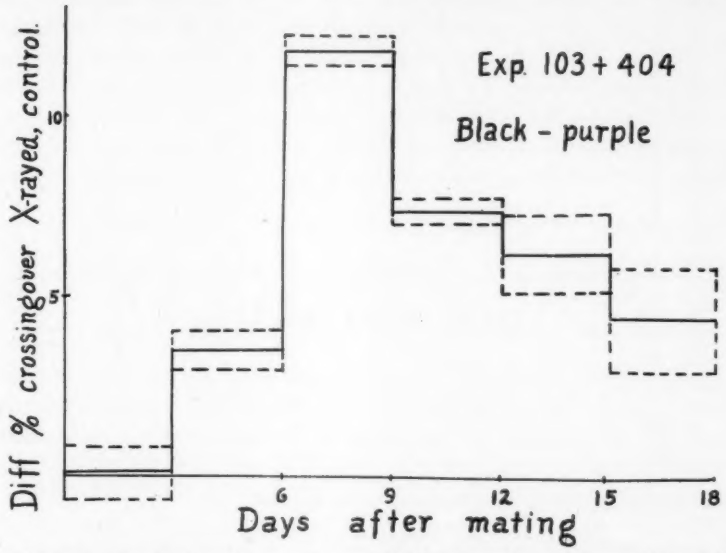

FIGURE 8.—Graph of crossing over between black and purple, experiments 103 and 404 being combined, plotted according to the method used in figure 2.

cannot be determined with a sufficiently small probable error to admit of an accurate comparison. The values found are shown in table 4. They show that there is no great increase or decrease in the coincidence due to the X-ray treatment.

TABLE 4

Experiment 404. Effect of X rays on coincidence of crossing over in the second chromosome, black to purple and purple to curved.

BOTTLE NUMBER.....	1	2	3	4
Coincidence, control...	149.6	180.5	114.3	91.5
Coincidence, X-rayed..	96.3	140.1	101.8	147.0

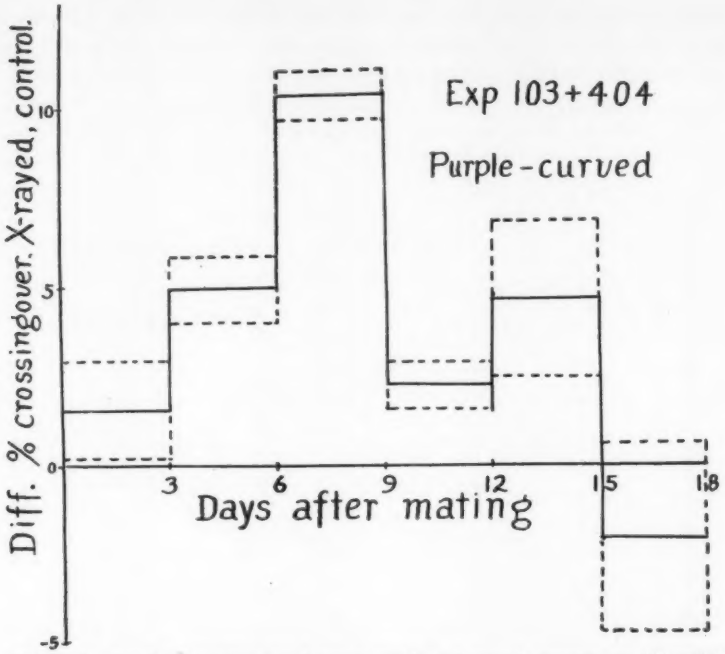

FIGURE 9.—Graph of crossing over between purple and curved, experiments 103 and 404 being combined, plotted according to the method of figure 2.

DISCUSSION

Viability

The experiments show that the crossover value, as determined from counts of the offspring, is greater in the case of females which were submitted to X-ray treatment than in the case of their sisters which did not receive the treatment but were otherwise reared under identical conditions. The question arises whether this increased crossover value found in the case of the X-rayed females is really an expression of an increased amount of crossing over in the eggs. That the X-ray treatment had an injurious effect on some of the eggs is shown by the lessened fertility of the X-rayed females. It is known, further, that the different mutant forms used in determining the crossover values have different viabilities. May it not be that the X-ray treatment merely accentuates the differences in viability between the crossover and non-crossover classes?

In considering this question it is to be noticed that the effect of the X

rays is to give an *increased* crossover value. It is clear that an increased crossover value which was due to greater differences in the viabilities of the classes of offspring could only be brought about by one of the three following conditions: (1) a relative decrease in the viability of one or more of the non-crossover classes; (2) an increase in the viability of one or more of the crossover classes; (3) a combination of these two conditions. BRIDGES and MORGAN (1919) have discussed the determination of the viabilities of the classes of offspring in experiments on crossing over. An accurate determination of the viabilities of the various crossover and non-crossover classes in the experiments now under consideration would require three additional experiments similar to either of those described but differing from them in having different groupings of the mutant genes in the chromosome. Thus, while in the experiments described, the females had the formula $\frac{+\ +\ +}{b\ p_r\ c}$, to determine the viability of the different classes experiments in which the females had the formulae, $\frac{b\ +\ +}{+\ p_r\ c}$, $\frac{b\ p_r\ +}{+\ +\ c}$, and $\frac{b\ +\ c}{+\ p_r\ +}$, would be required. It is possible, however, from the data at hand, to compare the viabilities of certain of the classes. Such a comparison is given in table 7, in which the numbers of offspring in the complementary classes are compared. Considering first the non-crossover classes it is seen that in both the cultures of the control and X-rayed females the number of offspring showing all three of the mutant characters is approximately equal to the number of normal offspring. Hence, if the increased crossover value shown by the X-rayed females, is to be attributed to a decreased viability of the non-crossover classes, the decrease must be the same whether the offspring are normal or contain the three mutant characters. The crossover classes may be considered next. In the case of crossover 1, that between black and purple, there is a noticeable difference between the viability of the $+p_r\ c$ class and the $b++$ class, the $b++$ class being the more viable, and this difference is greater in the case of the offspring of the X-rayed females. It might be conceived therefore that the increased crossover value found for the X-rayed females for the black-to-purple region was due to a *relatively* increased viability of the $b++$ and $+p_r\ c$ classes. It is necessary to include both of these classes, since, if the crossover value is found by taking only the $+p_r\ c$ class (the smaller) with one of the non-crossover class (either one, since they are approximately equal), the value found is still much greater (approximately three times in experiment 404) in the case of the X-rayed females than in that

of the control. Turning to the crossover 2, that between purple and curved, it is seen that here also the two classes of offspring do not show equal viability, but that the class, $++c$, is more viable. However, the relative viability, as shown by the ratio, is approximately the same in the offspring of the control and X-rayed females, so that here again if the increased crossover value is due to differences in viability, both the classes of offspring of the X-rayed females must show increased viability. In the case of the classes of double crossovers the numbers are probably too small to give definite information as to the relative viabilities of the classes. However, it can be shown, as in the two previous cases, that, if the increase in the double crossing over is to be accounted for by an increase in the viability of the classes of double crossovers, then the viability of each class must have been increased. An examination of the numbers of offspring in the crossover classes leads to the conclusion that if the increased crossover value shown by the X-rayed females is due to a relatively increased viability of the offspring in the crossover classes, then the increased viability must affect each of the classes of crossovers. In other words, in all cases where crossing over occurs, the offspring have or acquire greater resistance to X-ray injury. The alternative to this conclusion is that the effect of the X rays is to actually increase the amount of crossing over in the eggs. Between these two the experimental data do not give the means to decide, although it is difficult to see how merely going through the process of crossing over should increase the resistance of an egg to the effects of X rays.

Comparison of the effects of X rays on the first and second chromosomes

The effect of X rays on crossing over in the second chromosome may be compared with the effect of X rays on crossing over in the first or sex chromosome already recorded by one of us (MAVOR 1923 a, b). Here it was found that X rays decrease the crossover value for eosin-eyed and miniature-winged, the effect increasing with the dose. After a dose approximately the same as that given in the first of the experiments on the second chromosome the crossover value for eosin and miniature was decreased from approximately 25 percent to less than 10 percent and the effect continued from the sixth to the twelfth day after the treatment. We see in the effects which X rays produce on crossing over in the first and second chromosomes of Drosophila a remarkable instance of opposite effects on the mechanism of heredity produced by the same treatment with the same physical agent.

Comparison of the effects of X rays and temperature on crossing over

It is of great interest to compare the effect which the experiments described show X rays to have on crossing over in the second chromosome with the effect which PLOUGH (1917) found temperature to have on crossing over. PLOUGH found, using the same characters, that submitting females to temperature either considerably above or below the normal rearing temperature (22°C) caused an increase in the crossover value. The X-ray experiments show that X rays also cause an increase in the crossover value when the flies are kept at a temperature of 22°C. Whereas PLOUGH found that the effect of temperature on crossing over became first apparent on the seventh to eighth day after the beginning of the heat treatment, the X-ray experiments show that the X-ray effect on crossing over becomes apparent on the fourth to seventh day (first experiment) or third to sixth day (second experiment).[1] It has already been stated that X rays cause the death of a considerable number of the eggs in the later stages of maturation. It is possible that eggs which are in the earlier stages of the crossing-over process and survive may be accelerated in maturation by the X rays and thus be laid at an earlier period. This may offer an explanation of the difference in the time of the beginning of the increase in the crossover value in the experiments of PLOUGH (1917) and the present experiment. It should be stated, however, that there is no evidence of such acceleration, e.g., earlier hatching out of offspring of X-rayed flies. A more striking difference is that, while the effect of temperature lasts for a time corresponding to the period of time treated and then disappears abruptly, the effect of an X-ray treatment which lasted only 3 minutes and 15 seconds starts on the third to sixth day, reaches a maximum on the sixth to ninth day, and then gradually falls off, the effect being still evident after fifteen days.

Do X rays directly affect the process of crossing over?

The question is raised as to whether X rays affect directly or only indirectly the process of crossing over. A further comparison of the results of the X-ray experiments with those of PLOUGH (1917) gives evidence on this point. If PLOUGH's figures 5 and 7 be compared with our figures 1 and 3 it will be noted that in the case of his figure 5 (p. 177) where the heat treatment was begun on the day of hatching and continued for seven days, the rise in the crossover value appears suddenly in the counts of the seventh to ninth days and disappears as suddenly in the counts of the

[1] In a third experiment recently completed, the X-ray effect does not appear until the sixth day.

fifteenth to seventeenth days and in the case of his figure 7 (p. 178) where the heat treatment lasted from the third to the eleventh day the rise in percent of crossing over begins somewhat gradually in the counts of the ninth to eleventh days, rises to maximum in the counts of the fifteenth to seventeenth days and disappears abruptly in the counts of the nineteenth to twenty-first days. The tables and curves given by PLOUGH bring out the following facts (p. 181):

> "Exposure of a female to a temperature of 31.5°C for a given period results in a decided increase in the percentage of crossing over among her offspring for a period about as long as the exposure. In case she is exposed up to the time of hatching this increase shows among the offspring of the first seven or eight days after mating. If the adults are exposed, the effect begins to be noted at the seventh or eighth day after the adults first began to receive treatment. The increase is maintained for the definite period mentioned above only."

PLOUGH believes that

> "The facts admit of only one interpretation, namely, that the high or low temperature has an effect on the chromosomal mechanism at one point in the development of the eggs only, and that eggs which have already passed this particular point in their development when the new temperature is applied, or which do not reach that point during the period of exposure, register no higher percentage of crossing over than the controls."

In contrast to these results are the results of the X-ray treatment. Here it may be said that an X-ray treatment given to females after hatching and before mating leads to an increase in the crossover value of the black-to-purple region beginning from three to six days after the treatment, rising to a maximum on the sixth to ninth day, and lasting until twelve to fifteen days after the treatment. Since we are dealing here with the time during which eggs were laid, the parents having been transferred to new bottles every three days, it is not possible to attribute the prolongation of the effect of the X-ray treatment on the crossover value to retarded development of the eggs.

One is therefore forced to conclude that X rays do not affect the "chromosomal mechanism at one point in the development of the eggs only." The effect which X rays have in increasing the crossover values in the second chromosome is therefore seen to be due to some effect which they have either on the chromosomes or on some other part of the mechanism involved, which produces a condition which increases the amount of crossing over. X rays can not be said, therefore, to act directly on the process of crossing over in this case.

CONCLUSIONS

1. It has been shown that X-ray treatment of the female leads to an increase in the crossover values for black to purple and purple to curved.

2. The greatest effect for the same dose is produced in the black-to-purple region.

3. An X-ray treatment lasting for 3 minutes and 15 seconds causes an increase in the crossover value for the black-to-purple region lasting for at least twelve days.

4. A comparison of these results with those of PLOUGH (1917) leads to the conclusion that X rays do not directly affect the process of crossing over, but that they produce either in the chromosomes or some other part of the mechanism a condition which causes an increase in crossing over.

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APPENDIX—Tables 5 to 7

TABLE 5

Experiment 103. Effect of X rays on crossing over in the second chromosome. The columns give actual numbers of flies. The column headed 1 refers to the black-to-purple region, and the column headed 2, to the purple-to-curved region. The column headed 1-2 contains double crossovers.

BOTTLE		TOTAL	NON-CROSSOVER	1	2	1–2
First.....	Control	808	637	32	126	13
	X-rayed	12	11	—	1	—
Second.....	Control	1472	1192	63	188	29
	X-rayed	65	43	5	10	7
Third.....	Control	1299	1066	42	176	15
	X-rayed	201	110	34	42	15
Fourth.....	Control	1262	1027	31	191	13
	X-rayed	173	121	24	22	6
Fifth.....	Control	1199	981	29	179	10
	X-rayed	137	100	9	24	4
Sixth.....	Control	1556	1257	49	234	16
	X-rayed	93	73	7	12	1

TABLE 6

Experiment 404. Effect of X rays on crossing over in the second chromosome. The columns give actual numbers of flies. The column headed 1 refers to the black-to-purple region, the column headed 2 to the purple-to-curved region. The column headed 1-2 contains double crossovers.

BOTTLE		TOTAL	NON-CROSSOVER	1	2	1–2
First.....	Control	1030	807	31	180	12
	X-rayed	438	334	17	83	4
Second.....	Control	1411	1377	36	187	11
	X-rayed	671	516	37	108	10
Third.....	Control	1493	1254	46	185	8
	X-rayed	1505	982	168	302	53
Fourth.....	Control	1223	999	44	173	7
	X-rayed	2602	1912	227	408	55

TABLE 7

The comparative viability of the non-crossover and crossover classes in experiments 103 (second to sixth bottles) and 404 (second to fourth bottles). The figures are for black, purple and curved. + = wild character; − = mutant character.

	CLASS	EXPERIMENT 103		EXPERIMENT 404	
		Control	X-rayed	Control	X-rayed
Non-crossover.....	+++	1722	141	1875	1672
	−−−	1680	122	1755	1688
Ratio.....		1.02	1.16	1.07	0.99
Crossover in region 1	+−−	65	22	58	160
	−++	75	43	68	269
Ratio.....		0.87	0.51	0.85	0.60
Crossover in region 2	−−+	238	28	201	305
	++−	321	46	336	498
Ratio.....		0.74	0.61	0.60	0.61
Crossover in regions 1 and 2	−+−	32	25	12	79
	+−+	28	4	14	37
Ratio.....		1.14	6.3	0.86	2.1